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# Clinical Mycology

SECOND EDITION

Elias J. Anaissie  
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# Preface

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The first edition of *Clinical Mycology* established this text as an important, internationally recognized reference work for clinical mycology. Owing to numerous recent advances in the diagnosis and management of mycoses, a second edition is mandated to provide clinicians and laboratorians with a contemporary source of information. The second edition provides modern tools that will assist in the diagnosis, prevention and treatment of fungal infections in various patient populations. A group of internationally recognized experts was assembled to present this information in a comprehensive, authoritative manner with a clear focus on the clinical management of fungal infections.

All chapters in the second edition have been extensively revised and updated with current information and references. Obsolete and out-of-date material was eliminated to maintain this important textbook as a single volume. Two new chapters have also been added; one on pneumocystosis, in recognition of the reclassification of *Pneumocystis jiroveci* as a fungus and another covering anomalous fungal and fungal-like infections; that is, Lacaziosis and Rhinosporidiosis. Several new sections have been added to the chapter on fungal infections in cancer patients to reflect the formidable clinical challenges these infections continue to present.

The success of the first edition was due in part to the use of practical tools (algorithms, slides, graphs, pictorials, photographs, and radiographs) that have made the work clinically practical. A significant effort has been made in the second edition to enhance these reader-friendly features with a significant increase in the number of practical tools, algorithms and the expanded use of color for enhanced clarity.

The book is divided into 4 sections (32 chapters) covering the following topics:

- I. *General principles*, covering epidemiology, pathogenesis, immunology, diagnostics and antifungal therapy (7 chapters).
- II. *The organisms*, which includes a discussion of the pathogenesis, laboratory and clinical characteristics and treatment of infections caused by various fungal pathogens (11 chapters).
- III. *Clinical syndromes and organ systems*. In this section, fungal infections are discussed according to host characteristics (AIDS, cancer, solid organ transplantation and pediatric populations) and according to the organ(s) involved (e.g., respiratory tract, central nervous system, etc.) (12 chapters)
- IV. *Special considerations*. This section covers fungal infection associated with geographic location, travel and occupation and mycotoxicosis of relevance to humans (2 chapters).

We believe that the enormous efforts of all contributors to the second edition of *Clinical Mycology* have resulted in a state-of-the-art and clinically useful textbook that will guide clinicians in the diagnosis, prevention and treatment of fungal infections in various patient populations.

The editors wish to thank the remarkably talented authors who have contributed to *Clinical Mycology* and the superb Infectious Diseases team at Elsevier.

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2008

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# The epidemiology of fungal infections

Shawn R. Lockhart, Daniel J. Diekema, Michael A. Pfaller

Fungal infections may be divided into two categories: nosocomial and community associated. Nosocomial fungal infections are defined as those acquired in a healthcare setting, and are almost always *opportunistic* mycoses. In contrast, community-associated fungal infections include not only opportunistic mycoses but also the *endemic* mycoses, for which susceptibility to the infection is acquired by living in a geographic area constituting the natural habitat of a pathogenic fungus and possessing risk factors that are predisposing.

Over the past two and a half decades, the incidence of both nosocomial and community-associated fungal infection has increased dramatically. An analysis of trends in infectious disease mortality in the United States found that fungal infections had risen from the tenth to the seventh most common cause of infectious disease related mortality between 1980 and 1997.<sup>1</sup>

Numerous factors have contributed to the increase in fungal infections, most notably a growing population of immunosuppressed or immunocompromised patients whose mechanisms of host defense have been impaired by primary disease states (e.g., AIDS, cancer), a mobile and aging population with an increased prevalence of chronic medical conditions, and the use of new and aggressive medical and surgical therapeutic strategies, including broad-spectrum antibiotics, cytotoxic chemotherapies, and organ transplantation.

## Nosocomial fungal infections

### Increasing incidence and mortality

For the past two decades, hospitals have been experiencing increasing problems with nosocomial fungal infections.<sup>2-5</sup> A recent study of the epidemiology of sepsis found that the annual number of cases of sepsis caused by fungal organisms in the United States increased by 207% between 1979 and 2000.<sup>2</sup> In the Surveillance and Control of Pathogens of Epidemiological Importance Study, a 49-center study of 24,179 nosocomial bloodstream infections recorded between 1995 and 2002, 9.5% of the infections were fungal in origin.<sup>6</sup> *Candida* spp. were the fourth leading cause of nosocomial bloodstream infections, surpassed only by staphylococci and enterococci (Table 1-1).<sup>6</sup>

**Table 1-1** Nosocomial bloodstream infections: most frequent associated pathogens. Scope surveillance program, April 1995 to September 2002<sup>a</sup>

Rank	Pathogen	% of isolates <sup>b</sup>
1	Coagulase-negative staphylococci	31.3
2	<i>Staphylococcus aureus</i>	20.2
3	<i>Enterococcus</i> spp.	9.4
4	<i>Candida</i> spp.	9.0
5	<i>Escherichia coli</i>	5.6
6	<i>Klebsiella</i> spp.	4.8
7	<i>Pseudomonas aeruginosa</i>	4.3
8	<i>Enterobacter</i> spp.	3.9
9	<i>Serratia</i> spp.	1.7
10	<i>Acinetobacter baumannii</i>	1.3

<sup>a</sup>Data reproduced from Wisplinghoff et al.<sup>6</sup>  
<sup>b</sup>Percent of a total of 24,179 infections.

Rates of invasive fungal infection vary by hospital and region because they are dependent upon local factors and practice patterns as well as underlying risk factors. However, the first population-based incidence rates of fungal infection were provided by an active laboratory surveillance program conducted in the San Francisco Bay area between 1992 and 1993.<sup>7</sup> The cumulative incidence of invasive mycoses in this study was 178 per million population. The most common nosocomial fungal pathogens were *Candida* (73 cases per million per year), *Aspergillus* (12 cases per million per year), and zygomycetes (~2 cases per million per year) (Table 1-2).<sup>7</sup> *Cryptococcus* was also a major cause of invasive mycoses in this study (65 cases

**Table 1-2** Population-based incidence rates and case-fatality rates for opportunistic mycoses

Organisms <sup>a</sup>	No. cases per million per year <sup>b</sup>	Case-fatality ratio (%) <sup>b</sup>
<b>Yeasts</b>		
A. <i>Candida</i> species	72.8	33.9
<i>C. albicans</i>		
<i>C. glabrata</i>		
<i>C. parapsilosis</i>		
<i>C. tropicalis</i>		
<i>C. krusei</i>		
<i>C. lusitaniae</i>		
<i>C. rugosa</i>		
<i>C. guilliermondii</i>		
<i>C. inconspicua</i>		
<i>C. norvegensis</i>		
B. <i>Cryptococcus</i> species	65.5	12.7
C. Other yeasts		
<b>Hyaline moulds</b>		
A. <i>Aspergillus</i> species	12.4	23.3
B. Zygomycetes	1.7	30.0
C. Other hyalohyphomycetes	1.2	14.3
<b>Dematiaceous moulds</b>	1.0	0
<b><i>Pneumocystis jirovecii</i></b>		
<sup>a</sup> List not all-inclusive.		
<sup>b</sup> Data reproduced from Rees et al. <sup>7</sup>		
Table reproduced from Pfaller and Diekema. <sup>21</sup>		

per million per year), which reflected the large number of patients at high risk due to HIV infection in the era prior to highly active antiretroviral therapy (89% of the patients with cryptococcosis were also HIV positive).<sup>7</sup>

The increasing rates of invasive fungal infection have also resulted in significant mortality. In one report, the number of deaths in the United States in which mycosis was listed on the death certificate increased fourfold between 1980 (1557 deaths) and 1997 (6534 deaths).<sup>1</sup> The crude mortality of fungal infections ranges from 27% to 77% but may exceed 90% in certain patient populations (e.g., aspergillosis or fusariosis in bone marrow transplant patients with persistent neutropenia). Although estimates of attributable mortality are confounded by the serious underlying diseases in many of these patients, matched cohort studies have confirmed that the mortality directly attributable to the fungal infection is extremely high.<sup>8-10</sup> A retrospective cohort study of fungal infections in Italian patients with hematologic malignancies placed the attributable

mortality at 33% for candidemia, 42% for aspergillosis, 53% for fusariosis and 64% for zygomycosis.<sup>11</sup>

## Risk factors

Although numerous risk factors for nosocomial fungal infection have been identified (Table 1-3), most are common in hospitalized patients and thus may not be useful in predicting those individuals who will develop invasive mycosis.<sup>8,12,13</sup> In an attempt to control for confounding factors such as underlying illness, several studies have used multivariate analysis to identify independent risk factors such as antimicrobial use, administration of chemotherapy, presence of indwelling catheters, colonization at other body sites, and hemodialysis (see Table 1-3).<sup>8,13</sup> The various exposures place individuals at risk for fungal infection primarily by inducing immunosuppression, promoting colonization or providing direct access to the bloodstream, lung or deep tissues (see Table 1-3).

Among patients at highest risk of fungal infection are solid organ transplant (SOT) and hematopoietic stem cell transplant (HSCT) recipients (Tables 1-4 and 1-5). For SOT recipients, the type of organ transplanted may predispose a patient to one type of fungal infection over another (Table 1-4)<sup>14</sup> while for HSCT recipients, risk for fungal infection depends upon the degree of immunosuppression (e.g., higher for allogeneic than for autologous transplants).<sup>5,15,16</sup> Risk factors for fungal infections in transplant recipients include the use of large doses of corticosteroids, multiple or acute rejection episodes (SOT), graft-versus-host disease (HSCT), hyperglycemia, poor transplant function, leukopenia, and advanced age.<sup>17</sup>

## Pathogens

### *Candida* species

Although the array of fungal pathogens known to cause nosocomial infection is extremely diverse, most of these infections are due to *Candida* spp.<sup>6</sup> *Candida* spp. accounted for 88% of all nosocomial fungal infections in the United States between 1980 and 1990 and were the fourth leading cause of nosocomial bloodstream infection (BSI).<sup>5,12</sup> A more recent multicenter surveillance program found that *Candida* species caused over 70% of invasive fungal infections in hospitalized patients (Fig. 1-1).<sup>18,19</sup> Between 1995 and 2002, the frequency of nosocomial candidemia rose significantly from 8% to 12% of all reported BSIs.<sup>6</sup> Wenzel and Gennings, extrapolating from these data, estimate the annual burden of candidemia to be 10,500–42,000 infections in the United States, associated with between 2800–11,200 deaths per year.<sup>20</sup> National Hospital Discharge Survey (NHDS) data estimates of invasive candidiasis incidence have been steady or increasing between 1996 and 2003 at 22–29 infections per 100,000 population (Fig. 1-2).<sup>21</sup> These data include not only candidemia but also other forms of invasive candidiasis that may not be associated with positive blood cultures, which may partially explain why the estimates are higher than several recent population-based studies of candidemia incidence (Table 1-6).<sup>22-30</sup> Combined with data from the NNIS system, which show an overall decline in frequency of candidemia among ICU patients in the US,<sup>4</sup> these data suggest that the burden of invasive candidiasis is shifting from the ICU to the general hospital (and even outpatient) setting.



**Table 1-3** Risk factors for fungemia in hospitalized patients

Risk factor	Possible role in infection
Antimicrobial agents <sup>a</sup>	
Number	Promote fungal colonization
Duration	Provide intravascular access
Adrenal corticosteroid	Immunosuppression
Chemotherapy <sup>a</sup>	Immunosuppression
Hematologic/solid organ malignancy	Immunosuppression
Previous colonization <sup>a</sup>	Translocation across mucosa
Indwelling catheter <sup>a</sup>	
Central venous catheter	Direct vascular access
Pressure transducer/Swan–Ganz	Contaminated product
Total parenteral nutrition	Direct vascular access Contamination of infusate
Neutropenia (polymorphonuclear cells <500/mm <sup>3</sup> ) <sup>a</sup>	Immunosuppression
Extensive surgery or burns	Route of infection Direct vascular access
Assisted ventilation	Route of infection
Hospitalization or intensive care unit stay	Exposure to pathogens Exposure to additional risk factors
Hemodialysis <sup>a</sup>	Route of infection Immunosuppression
Malnutrition	Immunosuppression

<sup>a</sup>Independent risk factor.

The excess (or attributable) mortality due to *Candida* spp. bloodstream infection is high (20–50%), and two studies performed at the University of Iowa Hospital demonstrate that this mortality did not change substantially between 1983 and 2001.<sup>9,10</sup> In addition, among patients who survive an episode of candidemia, the mean excess length of stay in the hospital attributable to the infection is 30 days.<sup>9</sup> Population-based mortality burden due to invasive candidiasis is available from National Center for Health Statistics (NCHS) multiple cause

of death data, which reveal that the mortality associated with invasive candidiasis has remained steady since 1997 at approximately 0.4 deaths per 100,000 population (Fig. 1-3).<sup>21</sup>

Although more than 100 species of *Candida* have been identified, fewer than 20 species have been implicated in nosocomial infections. *C. albicans* is the species most commonly isolated from clinical material and accounts for 40–70% of cases of invasive candidiasis.<sup>21,23,31–35</sup> The second and third most frequently isolated species of *Candida* causing nosocomial candidiasis are dependent upon the age of the patient and the geographic location of the hospital (Table 1-7). In the NICU setting in the United States *C. parapsilosis* is the second most frequently isolated organism while in the general ICU setting it is *C. glabrata*. Despite reports suggesting that shifts have occurred in the distribution of infections caused by species of *Candida* other than *C. albicans*, many of these reports are isolated to specific institutions, and we have observed that the rank order of species distribution has been stable over 12 years of global surveillance.<sup>36</sup>

Accumulated knowledge about the epidemiology of nosocomial candidemia is summarized in Figure 1-4. Certain hospitalized patients are at increased risk of contracting nosocomial candidemia because of their underlying medical conditions, while medical interventions such as antibiotic use, the presence of a central venous catheter, and hemodialysis further increase the risk of contracting candidemia (Table 1-8).<sup>37</sup> The available epidemiologic data indicate that between 5 and 10 of every 1000 high-risk patients exposed to any of the preceding risk factors will contract *Candida* bloodstream infection, which comprises 8–10% of all nosocomial bloodstream infections.<sup>38</sup> Approximately 35% of these patients will die as a result of the infection, and an additional 30% will die because of their underlying disease.<sup>9</sup> In a recent matched cohort study of nosocomial candidemia, 49% of the patients died as a result of their infection while an additional 12% died of their underlying disease.<sup>10</sup>

Because delays in the administration of appropriate antifungal therapy are important contributors to the unacceptably high associated mortality, considerable efforts are now being made to develop risk stratification strategies to guide antifungal therapy (prophylaxis and early empiric therapy) to improve outcomes.<sup>21</sup>

### Aspergillus species

*Aspergillus* species are ubiquitous fungi that may be isolated from a variety of environmental sources, including soil, grain, leaves, grass, and air.<sup>39,40</sup> Reservoirs in hospitals from which aspergilli have been cultured include unfiltered air, ventilation systems, dust dislodged during construction, carpeting, food, and ornamental plants.<sup>39–42</sup> Although several hundred species of *Aspergillus* have been described, relatively few are known to cause disease in humans. *Aspergillus fumigatus* remains the most common cause of aspergillosis, although the proportion of aspergillosis cause by *A. fumigatus* has fallen from ~90% of cases in the 1980s to ~50–60% of cases in the 1990s into the 2000s.<sup>5</sup> The other species of *Aspergillus* commonly causing nosocomial infections include *A. flavus*, *A. terreus*, *A. niger*, *A. versicolor*, and *A. nidulans*.<sup>16,39,40,43</sup>

*Aspergillus* infections occur worldwide and appear to be increasing in prevalence.<sup>39,40</sup> National Hospital Discharge data from the 1990s reveal that there are approximately 10,000 aspergillus-related hospitalizations annually in the United States.<sup>44</sup>

**Table 1-4** Compiled incidence of fungal infections among organ transplant recipients, 1980–1999<sup>a</sup>

Organ transplant	Incidence of invasive fungal infection	Proportion of invasive fungal infection			
		Aspergillus	Candida	Cryptococcus	Other
Renal	0–20%	0–26%	76–95%	0–39%	0–39%
Heart	5–21%	77–91%	8–26%	NA	NA
Liver	4–42%	1–53%	35–91%	3–7%	3–15%
Lung and heart-lung	10–36%	20–50%	42–73%	18–26%	11%
Small bowel	33–59%	0–4%	80–100%	NA	0–11%
Pancreas and pancreas-kidney	6–38%	0–3%	97–100%	NA	NA
NA, data not available.					
<sup>a</sup> Adapted from Fungal infections. <sup>14</sup>					

**Table 1-5** Most common opportunistic mould infections in organ transplant and hematopoietic stem cell transplant recipients

Fungus	Percentage of invasive mould infections	
	SOT <sup>a</sup>	HSCT <sup>b</sup>
<i>Aspergillus fumigatus</i>	55%	51%
Other <i>Aspergillus</i> species	15%	26%
Non- <i>Aspergillus</i> hyalohyphomycetes and phaeohyphomycetes	18%	14%
Zygomycetes	6%	9%
Other	6%	-
<sup>a</sup> Data based on a multicenter study 1998–2002 by Husain et al. <sup>38</sup>		
<sup>b</sup> Data reproduced from Marr et al. <sup>5</sup>		

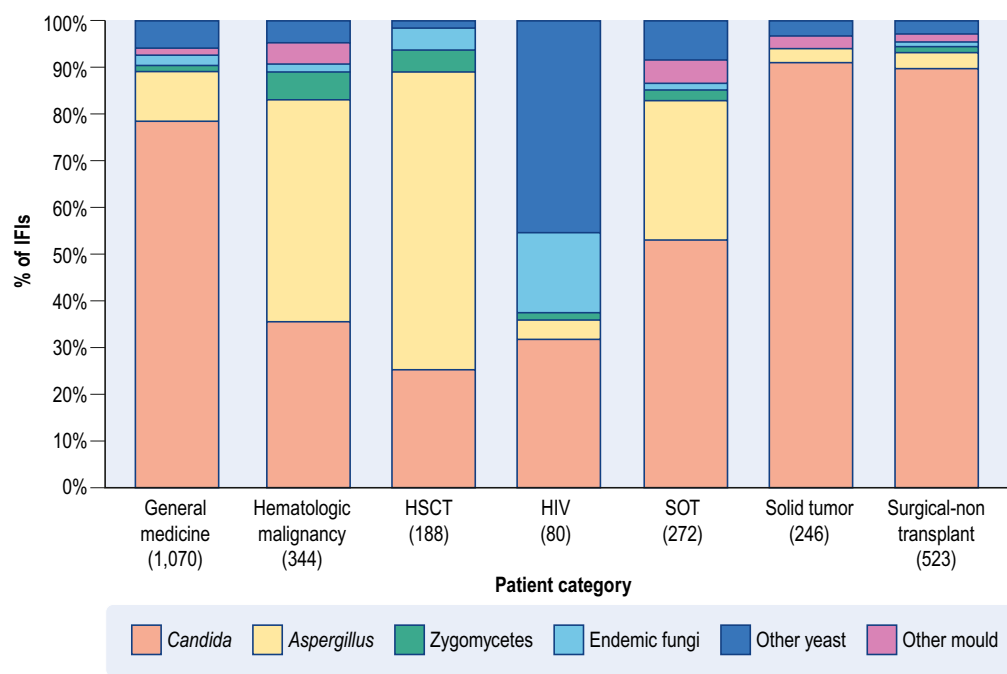
Although the total number of nosocomial infections due to *Aspergillus* spp. is small compared with those caused by *Candida* spp., *Aspergillus* spp. are particularly important causes of nosocomial infections in patients who are immunocompromised as a result of burn injury, malignancy, leukemia, and bone marrow and other organ transplantation.<sup>39,40</sup>

Although invasive aspergillosis is a devastating complication for SOT recipients,<sup>39</sup> the incidence of *Aspergillus* spp. infections in these patients has been lower than in HSCT recipients, probably because of the greater degree of granulocytopenia among HSCT recipients. Most studies place the cumulative incidence of invasive aspergillosis among allogeneic HSCT recipients at between 3% and 15%.<sup>16,45,46</sup> However, the incidence of aspergillosis increases in relation to the type

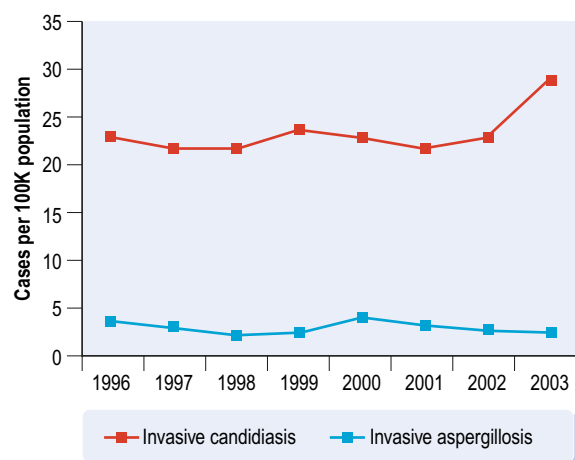
of donor used for transplantation (Table 1-9).<sup>16</sup> Major risk factors for invasive aspergillosis include neutropenia, broad-spectrum antibacterial therapy, administration of corticosteroids, antitumor necrosis factor therapy, and grade III–IV graft-versus-host disease (see Table 1-3).<sup>39,40</sup> The most important extrinsic risk factor is the presence of aspergilli in the hospital environment. Nosocomial transmission of *Aspergillus* to patients occurs primarily by the airborne route, but contact transmission (e.g., direct inoculation from occlusive materials) has also been implicated.<sup>41</sup> Outbreaks of nosocomial aspergillosis occur most commonly among granulocytopenic patients (<1000/mm<sup>3</sup>) and have been described in association with exposure to *Aspergillus* conidia aerosolized by hospital construction, contaminated air-handling systems, and insulation or fireproofing materials within walls or ceilings of hospital units.<sup>39–41</sup>

The crude mortality associated with invasive aspergillosis is high, but the attributable mortality has been difficult to determine given the high mortality rate in susceptible patients. A recent case review of nosocomial aspergillosis placed the attributable mortality rate at approximately 58%.<sup>47</sup> The highest attributable mortality rates have been observed among patients with aplastic anemia and after bone marrow transplantation. The survival rate of patients diagnosed with aspergillosis has been steadily increasing, especially in HSCT patients. The mortality rate in 1990 was >95% but by the end of that decade the mortality rate had decreased to between 55% and 80%.<sup>39</sup>

Prevention of nosocomial aspergillosis is a difficult issue and requires active surveillance for cases of aspergillosis, minimization of host risk factors, and maintenance of an environment as free as possible of *Aspergillus* spp. spores for patients with severe granulocytopenia.<sup>48</sup> For those at highest risk of invasive aspergillosis, provision of high-efficiency particulate air (HEPA) filtered environments is recommended.<sup>49</sup> Revised guidelines for prevention of nosocomial aspergillosis have been published by the CDC;<sup>48</sup> however, despite these efforts, invasive aspergillosis remains a constant threat to the survival of immunocompromised patients.



**Figure 1-1** Distribution of nosocomial invasive fungal pathogens based upon the underlying condition of the patient. HSCT, hematopoietic stem cell transplant; SOT, solid organ transplant. Data reproduced from Horn et al.<sup>18</sup> and Horn et al.<sup>19</sup>



**Figure 1-2** Incidence of invasive candidiasis and invasive aspergillosis in the United States, from NHDS, 1996–2003. Data reproduced from Pfaller and Diekema.<sup>21</sup>

### Zygomycetes

Zygomycosis is a general term that includes infections caused by fungi in the orders Mucorales and Entomophthorales (class Zygomycetes). *Rhizopus* species are the most frequent cause of zygomycosis but invasive infections in hospitalized individuals are also caused by species of *Mucor*, *Cunninghamella*, *Apophysomyces*, *Absidia*, *Saksenaia*, *Rhizomucor* and occasionally other representatives of this class of fungi.<sup>50</sup>

The zygomycetes are ubiquitous worldwide in decaying soil and vegetation, and infections may be acquired by inhalation, ingestion or contamination of wounds with conidia from environmental sources. The incidence of zygomycosis is on the rise. The Fred Hutchinson Cancer Center reported a doubling in the number of cases from 1985–1989 to 1995–1999,<sup>5</sup> and other reports have indicated an increase in incidence since

the 1990s.<sup>46,51</sup> Because many fatal cases of zygomycosis are diagnosed post mortem, it is difficult to establish the true incidence of this devastating disease, but one population-based study estimated the incidence at 1.7 cases per million people.<sup>7</sup> Zygomycosis accounted for 5.7% of opportunistic mould infections in solid organ transplant recipients.<sup>38</sup>

Nine hundred and twenty nine case reports of zygomycosis occurring between 1940 and 2003 were recently reviewed.<sup>50</sup> The most common sites of infection were sinus (39%), pulmonary (24%) and cutaneous (19%) with 23% of cases becoming disseminated. The overall mortality rate was high but varied according to the site of infection and the underlying condition of the patient. Risk factors for zygomycete infections include corticosteroid and deferoxamine therapy, diabetic ketoacidosis, hematologic malignancy, solid organ transplant, penetrating trauma or burns, and exposure to hospital construction activity.<sup>52,53</sup>

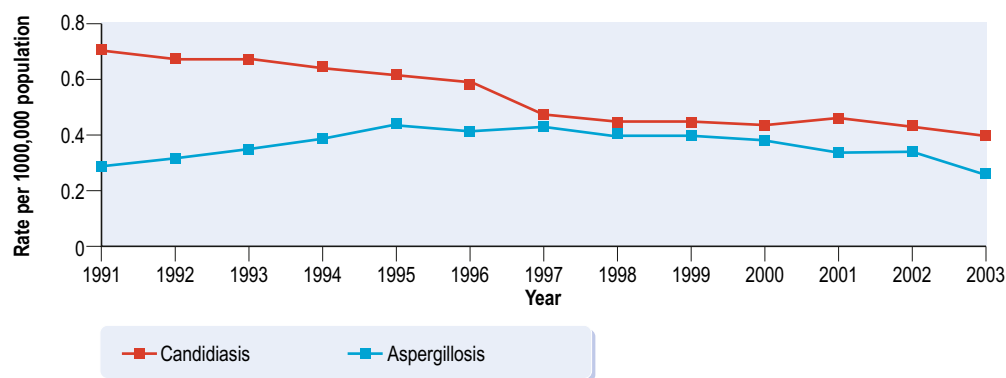
Recent case series<sup>54,55</sup> and one case-control study<sup>56</sup> suggest that exposure to voriconazole prophylaxis among HSCT recipients (used to prevent invasive aspergillosis) may be a risk factor for zygomycosis. While this phenomenon is still incompletely understood, it is clear that the use of broader spectrum antifungal agents for prophylaxis will inevitably change the epidemiology of fungal infections, and that selective advantage will accrue to those organisms with intrinsic resistance to the currently available antifungals.

### Emerging nosocomial fungal pathogens

Although most nosocomial fungal infections are caused by *Candida* and *Aspergillus* species, a significant number of infections are now caused by a diverse array of so-called emerging fungal pathogens. These organisms include yeasts other than *Candida* spp. or *Cryptococcus* spp., non-dematiaceous or hyaline moulds, and the pigmented or dematiaceous fungi (Table 1-10).<sup>57</sup> Infections caused by these organisms range from catheter-related fungemia and peritonitis to hematogenously

**Table 1-6** Estimated incidences of candidemia: population-based studies in Europe, Canada and the United States

Region	Year	Location	Cases/100K/yr	Reference
Europe	1995–1999	Finland	1.9	22
	1991–1994	Norway	2.0	23
	2001–2003	Norway	3.0	23
	1995–1999	Iceland	4.9	24
	2002–2003	Barcelona	4.9	25
	2003–2004	Denmark	11.0	26
United States	1998–2001	Iowa	6.0	27
	1992–1993	San Francisco, CA	7.1	28
	1992–1993	Atlanta, GA	8.7	28
	1998–2000	Connecticut	7.1	29
	1998–2000	Baltimore, MD	24.0	29
Canada	1999–2004	Calgary	2.8	30

**Figure 1-3** US crude mortality rates for invasive candidiasis and invasive aspergillosis, 1991 to 2003 (NCHS multiple cause of death data from public use files ([www.cdc.gov/nchs](http://www.cdc.gov/nchs))).

disseminated infections to more localized infections involving lung, skin, and paranasal sinuses.<sup>57</sup> The frequency of infections due to any one of these emerging pathogens is quite low, and thus our understanding of the epidemiology and modes of treatment for specific infections is minimal.

Among the non-*Candida* and non-*Cryptococcus* yeast pathogens, nosocomial infections due to *Malassezia* spp., *Trichosporon* spp., *Rhodotorula* spp., *Saccharomyces cerevisiae* and *Blastoschizomyces capitatus* (formerly *Geotrichum capitatum*) are most prominent (see Table 1-10). These rare pathogens may cause invasive (primarily bloodstream) infections in immunocompromised hosts, are usually associated with central venous catheters, and in many cases may demonstrate resistance to one or more antifungal agents. These infections are covered in more detail in Chapter 10, Infections caused by non-*Candida*, non-*Cryptococcus* yeasts.

The hyaline hyphomycetes constitute an array of fungal pathogens that are ubiquitous in the environment. As many as 20 different genera have been described as causative agents of hyalohyphomycosis, including such diverse opportunistic pathogens as *Acremonium*, *Chrysosporium*, *Fusarium*, *Paecilomyces*, *Penicillium*, *Scopulariopsis*, and *Sepedonium* species. Although infections caused by most of these fungi are relatively uncommon, they appear to be increasing in incidence.<sup>57</sup> Most disseminated infections are thought to be acquired by the inhalation of conidia or by the progression of previously

localized cutaneous lesions. The most important of these agents as a cause of nosocomial fungal infection is *Fusarium*.

*Fusarium* spp. have been recognized with increasing frequency as causes of nosocomial infection in immunosuppressed patients.<sup>5,38,58,59</sup> Patients with hematologic malignancies receiving cytotoxic chemotherapy, bone marrow transplant recipients, and patients with extensive burns are at increased risk for invasive fusariosis. *Fusarium* spp. was one of the three most common non-*Aspergillus* mould infections among hematopoietic stem cell recipients at the Fred Hutchinson Cancer Center between 1985 and 1999, with the most common species being the *F. solani* complex, *F. oxysporum* and *F. moniliforme*.<sup>5,60</sup> The outcome of disseminated fusariosis is dismal, with mortality between 79% and 87% at 90 days following diagnosis.<sup>59</sup>

Phaeohyphomycosis is defined as tissue infection caused by dematiaceous (pigmented) hyphae or yeasts. The dematiaceous fungi that have been documented to cause human infection encompass a large number of different species; however, most infections have been caused by *Alternaria*, *Bipolaris*, *Curvularia*, *Cladosporium*, *Exserohilum* and *Scedosporium* species (although the latter can also be recognized as an agent of hyalohyphomycosis). Risk factors for disseminated phaeohyphomycosis include immunosuppression, malignancy, neutropenia and leukemia.<sup>61</sup> A recent literature review of 72 cases of disseminated phaeohyphomycosis revealed *Scedosporium*



**Table 1-7** *Candida* species distribution from global and regional surveillance programs<sup>a</sup>

	<i>C. albicans</i>	<i>C. glabrata</i>	<i>C. tropicalis</i>	<i>C. parapsilosis</i>	<i>C. krusei</i>	Others
<b>All ages</b>						
United States 1993–1995	48%	24%	19%	7%		2%
United States 1989–1999	59%	12%	10%	11%	1%	7%
North Am. 2001–2004	51%	22%	7%	14%	2%	4%
Taiwan 1994–2000	50%	12%	21%	14%		3%
Asia 2001–2004	56%	10%	14%	16%	2%	2%
Norway 1991–2003	70%	13%	7%	6%	2%	2%
Spain 2001–2006	45%	12%	10%	19%	5%	9%
Europe 2001–2004	60%	10%	9%	12%	5%	4%
South Am. 1997	41%	2%	12%	38%		7%
Latin Am. 2001–2004	50%	7%	20%	16%	2%	5%
Brazil 2003–2004	41%	5%	21%	21%	1%	11%
<b>Children</b>						
United States 1993–1995	63%	6%		29%		2%
United States 1995–2004	58%	2%	4%	34%		2%
Slovakia 1998–2000	50%	7%	12%	17%	7%	7%

<sup>a</sup>Data reproduced from references 4, 21, 23, 31–33, 35.

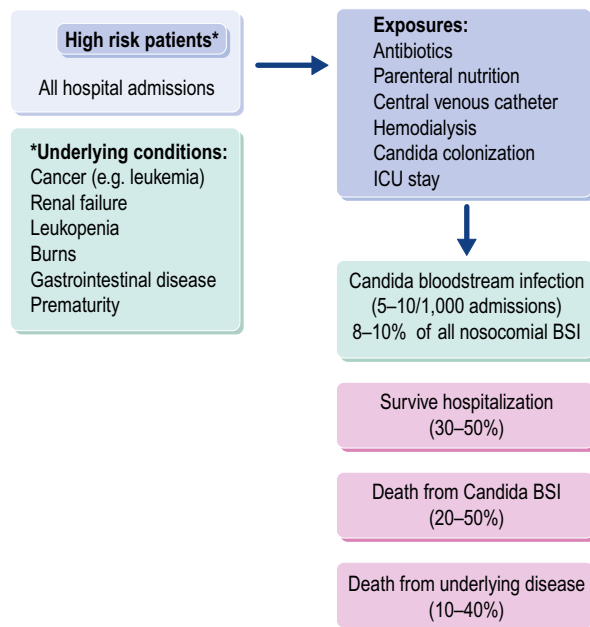
*prolificans* and *Bipolaris spicifera* to be the most commonly reported causes of disseminated disease and revealed that the outcome of antifungal therapy remains poor with a 79% overall mortality rate for this group of organisms.<sup>61</sup>

## Molecular Epidemiology: Reservoirs and Modes of Transmission

Modern epidemiologic studies now require that nosocomial pathogens be characterized below the subspecies level to better define infectious processes and modes of transmission.<sup>62,63</sup> Although many physiologic and protein-based typing methods

have been used in epidemiologic studies of fungal infection, the DNA-based molecular typing (DNA fingerprinting) methods have been most useful for this purpose.<sup>64</sup>

Molecular typing systems are used to assist the microbiologist, clinician, and epidemiologist in addressing the question of whether two or more isolates of a given species of fungus are “the same” or “different.” This question may arise in epidemiologic investigations, in the management of patients, or in studies of pathogenesis. A variety of typing methods have been used to provide molecular fingerprints of different fungi, and the method used in a given study may vary with the organism and the specific goals of the study (Table 1-11).



**Figure 1-4** Global view of hospital-acquired candidemia.

**Table 1-8** Factors for increased risk of high-risk patients contracting candidemia in the hospital setting compared to control subjects without specific risk factors or exposures<sup>a\*</sup>

Risk factors	Fold increased risk
Each class of antimicrobial received	2x
Patient has a central venous catheter	7x
<i>Candida</i> colonizes another site	10x
Patient has undergone acute hemodialysis	18x

\*Hospitalization in an ICU is an independent risk factor.  
<sup>a</sup>Adapted from Wenzel and Gennings.<sup>20</sup>

In a typical epidemiologic investigation, isolates from two or more patients are examined to determine whether the infections being studied are due to the same strain or due to different strains. In general, if isolates are classified as different by at least one molecular typing method, they may be assumed to represent different strains and to reflect independent infections.<sup>62,64</sup> If the isolates are the same, it may be assumed that cross-infection has occurred or that the patients were infected by exposure to a common source. The strength of these assumptions depends on the reproducibility and discriminatory power of the typing method used.<sup>62,64</sup> Typing methods may also be used to address clinical problems related to distinguishing reinfection versus relapse of an infection and to examine the development of antifungal resistance among fungal pathogens during the course of antifungal therapy. Multiple isolates obtained sequentially from an individual

**Table 1-9** Aggregate cumulative incidence of aspergillosis 12 months after transplantation<sup>a</sup>

Type of transplant	Rate of aspergillosis
After autologous HSCT	0.5%
After allogeneic HLA-matched donor transplant	2.3%
After HLA-mismatched donor transplant	3.2%
After unrelated donor transplant	3.9%

<sup>a</sup>Data reproduced from Morgan et al.<sup>16</sup>

patient may be tested to detect strain relatedness. Repeated infections with different strains of an organism may suggest that the patient is predisposed to that particular infection as a result of specific exposures or host defects, whereas recovery of the same strain on multiple occasions suggests a relapsing infection, possibly due to a residual focus such as an indwelling catheter or persistent colonization.<sup>64,65</sup> Likewise, determination of DNA fingerprints of sequential isolates from patients undergoing antifungal therapy has been useful in demonstrating the potential for the development of antifungal resistance in previously susceptible strains and for detecting the substitution of a more resistant strain for a more susceptible strain in the face of intense antimicrobial pressure.

DNA fingerprinting of fungal pathogens may be accomplished with a variety of different techniques (see Table 1-11). In almost all cases, DNA fingerprinting methods involve comparisons of patterns that are assumed to reflect genetic relatedness and are generated by some form of electrophoresis or DNA sequencing. To be useful as an epidemiologic typing method, a DNA fingerprinting system must effectively distinguish between genetically unrelated strains, be capable of identifying the same strain in separate samples, and reflect genetic relatedness or unrelatedness (genetic distance) among strains or species.<sup>64</sup> Although the ability of most of the DNA fingerprinting methods listed in Table 1-11 to measure genetic distance has not been established, qualitative analysis of the various DNA profiles has been useful in studies of several nosocomial fungal pathogens.<sup>64</sup>

One of the most recent developments in typing of the infectious fungi is multilocus sequence typing (MLST). This methodology was developed for bacterial population genetics and involves the amplification and sequencing of small portions of a number of housekeeping genes in search of stable sequence variations.<sup>66,67</sup> MLST allows for universal protocol development and strain archiving in large data sets, and eliminates interlaboratory variation, as long as a good sequencing reaction can be obtained. The single caveat to this protocol is that it may be cost prohibitive to laboratories without access to inexpensive sequencing. MLST protocols have been developed for *C. albicans*,<sup>68,69</sup> *C. glabrata*,<sup>70</sup> *C. tropicalis*,<sup>71</sup> *C. krusei*,<sup>72</sup> *Cryptococcus neoformans*,<sup>73</sup> and *Fusarium oxysporum*.<sup>74</sup> In addition, there is an online database listed for *A. fumigatus* ([www.mlst.net](http://www.mlst.net) and <http://pubmlst.org>).

**Table 1-10** Emerging nosocomial fungal pathogens

<b>Yeasts other than <i>Candida</i> and <i>Cryptococcus</i></b>
<i>Malassezia</i> spp. <i>M. furfur</i> <i>M. pachydermatis</i> <i>Trichosporon beigeli</i> <i>Rhodotorula rubra</i> <i>Saccharomyces cerevisiae</i>
<b>Hyalohyphomycetes</b>
<i>Fusarium</i> spp. <i>Acremonium</i> spp. <i>Paecilomyces lilacinus</i>
<b>Phaeohyphomycetes</b>
<i>Alternaria</i> spp. <i>Scedosporium prolificans</i> <sup>a</sup> <i>Scedosporium apiospermum</i> <sup>a</sup> <i>Bipolaris spicifera</i>
<sup>a</sup> May also be classified under hyalohyphomycetes.

Strategies for prevention and control of nosocomial fungal infections must take into account both endogenous and exogenous reservoirs for infection. In addition to the fact that colonization with a fungus frequently precedes infection, evidence for an endogenous source of nosocomial yeast infection includes the isolation of patient-unique strains from multiple anatomic sites over time and the fact that colonizing and infecting strains usually share the same DNA fingerprint profile.<sup>75,76</sup> Conversely, most nosocomial mould infections are acquired exogenously from the environment. Although molecular typing methods have been used infrequently to study nosocomial mould infections, Girardin et al<sup>77,78</sup> used a moderately repetitive DNA probe to fingerprint isolates of *A. fumigatus*. These investigators detected multiple genotypes of *A. fumigatus* in the hospital environment and found evidence for an environmental origin of a strain infecting two patients. Evidence exists to support the exogenous acquisition of other fungal pathogens as well.<sup>75,79</sup> Numerous accounts now exist of the transmission of *Candida* spp., *Malassezia* spp., *Pichia anomala*, *Exophiala jeanselmei*, *Saccharomyces cerevisiae* subtype *boulardii*, and *Trichosporon* to high-risk patients by means of contaminated infusates, biomedical devices or the hands of healthcare workers.<sup>75,79-82</sup> Studies of the inanimate hospital environment suggest that strains of *Candida* may survive on environmental surfaces,<sup>79</sup> that strains of *Aspergillus* may colonize decorative plants in the hospital, and that nosocomial acquisition of such strains may be documented.<sup>42,79</sup> As with endogenous infection, the epidemiology of exogenous acquisition of nosocomial fungal pathogens has been clarified by the application of molecular typing methods to identify common strains among isolates from exogenous sources and infected patients.

**Table 1-11** Molecular methods for epidemiologic typing of fungal pathogens

<b>Method</b>	<b>Fungal pathogens</b>
<b>DNA-based methods</b>	
Southern hybridization analysis (RFLP)	<i>Candida</i> spp. <i>Aspergillus</i> spp. <i>Cryptococcus neoformans</i> <i>Trichosporon beigeli</i> <i>Histoplasma capsulatum</i>
Restriction endonuclease analysis of genomic DNA (ethidium bromide)	<i>Candida</i> spp. <i>Aspergillus</i> spp. <i>Malassezia</i> spp. <i>Histoplasma capsulatum</i>
Pulsed-field gel electrophoresis	
Electrophoretic karyotyping	<i>Candida</i> spp. <i>C. neoformans</i>
Restriction endonuclease Digestion with rare cutters	
PCR fingerprinting	<i>Candida</i> spp. <i>Aspergillus</i> spp. <i>C. neoformans</i> <i>Histoplasma capsulatum</i> <i>Pneumocystis jiroveci</i>
<b>Multilocus sequence typing</b>	
	<i>Candida</i> spp. <i>C. neoformans</i> <i>A. fumigatus</i> <i>Fusarium oxysporum</i>
<b>Protein-based methods</b>	
Immunoblot fingerprinting	<i>Candida</i> spp. <i>Aspergillus</i> spp.
Polyacrylamide gel electrophoresis of cellular proteins	<i>Candida</i> spp.
Multilocus enzyme electrophoresis	<i>Candida</i> spp. <i>C. neoformans</i>

## Community-associated fungal infections

The agents of community-associated mycoses include the geographically delimited endemic dimorphic fungi and an ever-increasing array of opportunistic yeasts and moulds (Table 1-12). Despite tremendous differences in their individual physiologic and biologic characteristics, these organisms often originate extrinsically in the environment and share a

**Table 1-12** Agents of community-associated mycoses**Endemic dimorphic pathogens**

*Blastomyces dermatitidis*  
*Penicillium marneffe*  
*Histoplasma capsulatum*  
*Coccidioides immitis* complex  
*Paracoccidioides brasiliensis*

**Opportunistic pathogens**

*Candida* and other opportunistic yeasts

*Candida* spp.  
*Cryptococcus neoformans*  
*Trichosporon* spp.  
*Rhodotorula* spp.  
*Saccharomyces cerevisiae*

Hyalohyphomycetes

*Aspergillus* spp.  
*Fusarium* spp.  
*Scopulariopsis* spp.  
*Trichoderma* spp.

Zygomycetes

*Absidia* spp.  
*Mucor* spp.  
*Rhizomucor* spp.  
*Rhizopus* spp.

Phaeohyphomycetes

*Alternaria* spp.  
*Bipolaris* spp.  
*Curvularia* spp.  
*Exserohilum* spp.  
*Scedosporium prolificans*<sup>a</sup>  
*Scedosporium apiospermum*<sup>a</sup>

*Pneumocystis jiroveci*

**Subcutaneous pathogens**

*Sporothrix schenckii*

Agents of chromoblastomycosis

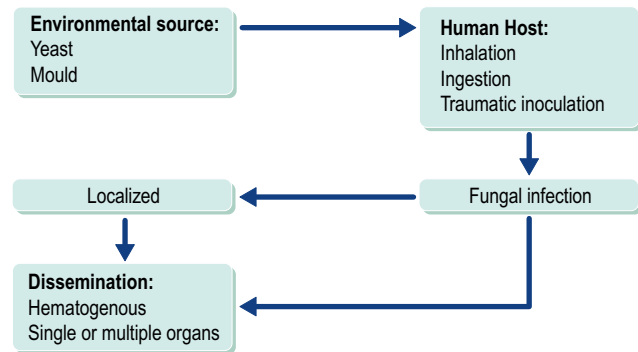
*Cladosporium* spp.  
*Fonsecaea* spp.  
*Phialophora* spp.

Agents of mycetoma

*Pseudallescheria boydii*  
*Madurella grisea*

<sup>a</sup>May also be classified under hyalohyphomycetes.

similar natural history with respect to human infection (Fig. 1-5). The infectious propagule, present in the environment as either a yeast or a mould, enters the human host by inhalation, ingestion or traumatic inoculation, and a localized infection is initiated in the lung, paranasal sinus or tissues. The extent of localized infection or dissemination to other organs largely depends on the infectious dose, the immune status of the host, and in some cases the specific properties of the infecting organism. Several of these community-associated mycoses

**Figure 1-5** Natural history of community-acquired (endemic and opportunistic) fungal pathogens.

produce serious, life-threatening disease, especially in individuals with immunocompromising conditions.<sup>17,83</sup>

**Endemic, dimorphic fungi**

Unlike nosocomial and community-associated infections caused by other opportunistic fungal pathogens, infections caused by the endemic, dimorphic pathogens *Histoplasma capsulatum*, *Coccidioides immitis* complex, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis* and *Penicillium marneffe* are acquired in specific geographic regions of the world.<sup>84,85</sup>

*Histoplasma capsulatum* var. *capsulatum* is the causative agent of histoplasmosis and is endemic to the central United States and Latin America. Serious infection with *H. capsulatum* is observed most commonly among individuals with AIDS and recipients of organ transplantation.<sup>17</sup> Histoplasmosis occurs in approximately 0.4% of renal transplant recipients<sup>86</sup> and in 2–5% of patients with AIDS from areas of endemicity. The organism is typically isolated from soil contaminated with avian or bat guano, and a number of epidemics have been associated with disruption of contaminated soil and with construction work in and around hospitals.<sup>87</sup>

Coccidioidomycosis, a disease caused by the dimorphic fungi *Coccidioides immitis* and *Coccidioides posadasii*, is endemic to the desert southwestern United States, northern Mexico, and Central and South America. The two species of *Coccidioides* are closely related and the description of *C. posadasii* as a separate species is relatively recent.<sup>88</sup> The major difference in species is in geographic range, with *C. immitis* limited primarily to California and *C. posadasii* also endemic in Texas, Arizona, Central and South America. *C. immitis* complex is found in soil, and the growth of the fungus in the environment is enhanced by bat and rodent droppings. Exposure to the infectious arthroconidia is heaviest in late summer and fall when dusty conditions prevail. Severe drought followed by periods of heavy rainfall have been associated with an excessive number of cases in recent years.<sup>89-91</sup> Acquisition of coccidioidomycosis occurs principally by inhalation of fungal arthroconidia, and in endemic areas infection rates may be 16–42% or greater by early adulthood.<sup>92</sup> Infection with *C. immitis* complex is a major threat to persons with AIDS and recipients of solid organ transplants who have resided in, currently reside in or have traveled to areas of endemic infection at any time during their life. Symptomatic coccidioidomycosis has been shown



**Table 1-13** Incidence of cryptococcosis prior to and following the era of highly active antiretroviral therapy in HIV/AIDS patients

Location	Incidence pre-HAART	Incidence post-HAART	Decrease
Atlanta, GA	66 cases/1000 patients 1992	7 cases/1000 patients 2000	89%
Houston, TX	24 cases/1000 patients 1993	2 cases/1000 patients 2000	92%
France	1352 cases 1985–1996	292 cases 1997–2001	46%

to occur in 4–9% of heart transplant recipients and 4–7% of renal transplant recipients in endemic areas.<sup>93</sup>

Although the geographic distribution of *Blastomyces dermatitidis* and *Paracoccidioides brasiliensis* is well defined, infections due to these organisms are relatively infrequent, and little is known of the incidence of infection. Both *B. dermatitidis* and *P. brasiliensis* may be acquired by contact with soil and organic material.<sup>94,95</sup> It is not clear that infection with *B. dermatitidis* or *P. brasiliensis* occurs with increased frequency among immunocompromised individuals, but one study noted that African-Americans may have an increased risk for blastomycosis.<sup>96</sup>

*Penicillium marneffei* is a recently recognized dimorphic fungus that is endemic in Southeast Asia. Although infections have been reported in both normal and immunocompromised hosts, most of the cases occur in HIV-infected individuals.<sup>97</sup> Thus far, all known infections have occurred in patients who have either lived in or traveled to Southeast Asia. In northern Thailand, infection with *P. marneffei* is the third most common opportunistic infection (after tuberculosis and cryptococcosis) among HIV-infected individuals.<sup>97,98</sup> The environmental reservoir for the organism appears to be two species of rat and their burrows. Although the fungus does not seem to exist in other parts of the world, increasing international travel makes it likely that infections will be detected far beyond the endemic range of the species.

## Opportunistic pathogens

The risk factors for community-associated opportunistic fungal infections include many of those listed in Table 1-4. In fact, in many instances, the distinction between nosocomial and community-associated opportunistic mycoses is not readily apparent. Increasingly, highly immunocompromised individuals are cared for in the home environment rather than the hospital and thus are exposed to fungal pathogens that they may or may not have encountered in the hospital environment.

Among the community-associated opportunistic fungal pathogens, the single most common agent of serious infection is *Cryptococcus neoformans*.<sup>99,100</sup> A rare disease before the onset of the HIV epidemic, cryptococcosis soon became a common cause of meningitis at many large hospitals caring for AIDS patients. Although precise estimates of the incidence of cryptococcal disease are not available, the incidence increased at least fivefold from 1980 to 1989 with a concomitant shift from older age groups before the advent of AIDS to the age groups most affected by HIV.<sup>99</sup> With the introduction of highly active antiretroviral therapy (HAART) the frequency of cryptococcal infection among HIV-positive persons has decreased significantly (Table 1-13).<sup>101,102</sup> HIV-associated deaths caused

by cryptococcosis have declined steadily since 1989 but showed a dramatic decrease between 1996 and 1997,<sup>1</sup> after the introduction of HAART. Despite the reduction in cryptococcosis since the introduction of HAART, it remains an important pathogen in this patient population.

*Cryptococcus neoformans* exists in two varieties, *neoformans* and *gattii*, which inhabit different ecologic niches. *C. neoformans* var. *neoformans* is found worldwide, most frequently from soil contaminated with bird guano. *C. neoformans* var. *gattii* is largely restricted to tropical and subtropical areas, and its major ecologic niche appears to be eucalyptus trees.<sup>103</sup> More recently, *C. neoformans* var. *gattii* has emerged as a cause of infection in British Columbia and the Pacific Northwest among individuals with no travel to *C. neoformans* var. *gattii* endemic areas.<sup>104</sup> Although the nature of the infectious particle for either variant is unknown, it is assumed that infection is acquired by inhalation of infectious forms from the environment.

*Pneumocystis jiroveci* (formerly *P. carinii*), another opportunistic fungal pathogen, was formerly classified as a protozoan. In the pre-HIV era, *P. jiroveci* was a relatively uncommon cause of pneumonia in immunocompromised hosts.<sup>105</sup> The HIV epidemic resulted in the emergence of *P. jiroveci* from a rare disease to a common cause of pneumonia (*Pneumocystis* pneumonia or PCP). PCP was the leading AIDS-defining illness among the HIV infected, affecting up to 75% of HIV-infected persons during their lifetime.<sup>106</sup> A decline in PCP incidence occurred after the introduction of PCP prophylaxis in 1989.<sup>107</sup> Later, the introduction of HAART further reduced rates of PCP.<sup>108</sup> However, since *P. jiroveci* is a ubiquitous environmental organism worldwide, infection rates remain high in areas of the developing world where access to prophylaxis and HAART is limited.<sup>109</sup>

## Conclusion

Infections due to both common and previously obscure or unusual fungi are being seen more frequently in both the hospital environment and the community. Unfortunately, our understanding of the epidemiology of fungal infections remains quite rudimentary and is hampered by inadequate diagnostic methods and the lack of mandatory reporting of fungal disease. The epidemiology of fungal infections is in a constant state of flux. Since the 1980s we have witnessed an increase in the incidence of aspergillosis in patients with hematologic malignancies while candidiasis decreased in the same group; a change in the species causing aspergillosis, with *A. fumigatus* on the decline and *A. terreus* on the rise; an increase in the incidence of non-*albicans* candidiasis; a general rise in the incidence of zygomycosis; and a dramatic decrease in

cryptococcosis among HIV/AIDS patients receiving HAART. Concentrated efforts by the CDC and other groups to study nosocomial fungal infections have increased our understanding of these important infections. These studies have been aided by the use of molecular typing methods. The shift in health-care from hospital-based care to outpatient-based care places greater emphasis on the need to understand the epidemiology of community-associated mycoses. Continued epidemiologic and laboratory investigation is needed to better characterize the ever-increasing array of endemic and opportunistic fungal pathogens, allowing for improved diagnostic, therapeutic, and preventive strategies in the future.

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# Recent advances in understanding human opportunistic fungal pathogenesis mechanisms

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## Introduction: fungal pathogens

Fungi are important components of the ecosystem. Fungi drive the global carbon cycle, help sustain agricultural and plant biodiversity through mycorrhizal associations, and provide mankind with other benefits including food, beer, and life-saving antibiotics.<sup>1</sup> The earliest fossil evidence of fungi is directly associated with plants (arbuscular mycorrhizae) and is approximately 460 million years old.<sup>2,3</sup> At some point in their evolutionary history, fungi evolved the ability to become pathogenic and utilize living organisms as sources of nutrients to complete their life cycles. Recent phylogenetic analyses of the fungi indicate that numerous transitions between pathogenic and saprophytic lifestyles have occurred.<sup>4</sup> It seems clear that the development of fungal plant pathogenesis was the result of a long co-evolutionary history between fungi and plants.<sup>5,6</sup>

This co-evolutionary relationship between fungi and plants is exemplified in the gene-for-gene hypothesis first observed in 1947 by H.H. Flor. Flor observed that corresponding genes in the pathogen and host determined outcomes of fungal-plant interactions. He proposed a model whereby a dominant resistance gene (*R* gene) in the host confers resistance against a fungal pathogen with a corresponding avirulence gene (*Avr* gene). Strains of the pathogen that lacked the dominant *Avr* gene could cause disease, until random mutation brought about another dominant *R* gene in the plant population that could provide resistance.<sup>7</sup> Viewed over time, an evolutionary “arms race” occurs where a random mutation in the fungal population leads to the arrival of a new *Avr* gene undetectable by the plant population resulting in disease, which persists until a new *R* gene appears in the plant population to provide resistance. Though this model has proved to be more complex than these single gene product interactions suggest, it is now well established that plant pathogenic fungi have evolved specific mechanisms to invade, elude, and overcome plant defense responses.<sup>8-10</sup>

In contrast to fungal plant infections, fungal infections of mammals are a relatively rare occurrence. This strongly suggests that mammals have evolved complex defense mechanisms against fungi and, importantly, that fungi have been unable to develop pathogenesis mechanisms to counteract mammalian resistance. Recently, it has been hypothesized that

the emergence of mammals at the end of the Cretaceous period is related to the ability to survive exposure to massive fungal challenges which may have occurred during this period.<sup>11</sup> Regardless, it is clear that mammals are remarkably resistant to fungi despite daily exposure to fungal conidia and hyphae. Understanding these mechanisms and how they prevent fungal disease is critical to our understanding of fungal pathogenesis in humans.

The mechanisms of this remarkable resistance are likely due to several inherent attributes of mammals. First, the core body temperature of most mammals is between 37°C and 39°C. The overwhelming majority of fungi thrive at temperatures between 25°C and 35°C and thus growth inside a mammalian host is not permissive for most fungi. Second, mammals have alkaline body fluids and most fungi prefer growth at a slightly acidic to neutral pH. Third, mammals have evolved complex innate and adaptive immune systems that prevent fungal growth from occurring when fungal conidia are inhaled. The combination of these three factors presents a formidable barrier to fungal infections in humans. In order for fungal infections to occur in humans, fungi must be able to overcome these barriers. Thus, this chapter ultimately deals with how fungi overcome these barriers to colonization and cause disease in mammalian hosts.

Recent events in modern medicine are dramatically changing the paradigm of fungi-mammal interactions. Advances in medical therapies, organ transplantation, HIV infections, and an increasing geriatric population have all resulted in a significant increase in life-threatening human fungal infections over the last two decades.<sup>12</sup> These underlying diseases and technologies have created increasing populations of immunocompromised patients susceptible to certain fungi that can overcome the innate factors of temperature, structural, and chemical barriers to infection found in mammals.

Fungal infections in immunocompromised patients are usually termed “opportunistic” as the fungi that most often cause these infections are saprophytic organisms not capable of causing disease in immunocompetent hosts. However, the recent rise of these lethal human fungal infections has led to a concerted effort to better understand fungal pathogenesis mechanisms in mammals. Current research efforts on fungal pathogenesis mechanisms in mammals have focused on

whether these opportunistic pathogens have evolved specific virulence factors to cause disease like their plant-pathogenic counterparts or whether they simply are well adapted to a host environment devoid of innate and adaptive immune responses. Recent technologic advances in molecular biology and genomic-based sciences have started to provide answers to this important question.

The purpose of this chapter is to review recent advances in our understanding of how important opportunistic fungal pathogens are able to cause disease in mammalian hosts, focusing on how an understanding of the biology of these organisms is critical to elucidating human fungal pathogenesis mechanisms. However, given the dramatic advances in this field over the last decade, this review will not be comprehensive for each fungus discussed; rather, we will seek to discuss some examples at the molecular level that illustrate how opportunistic fungal pathogens may be causing disease in immunocompromised patients. At the conclusion we will briefly touch on important issues for consideration on the host side of the infection.

## Definitions: what is a “virulence factor?”

A review of fungal pathogenesis mechanisms must first define the important yet controversial terms “virulence” and “pathogenesis.” The microbial pathogenesis literature is rich with debate on the definitions of these often misused terms. Yet, a clear understanding of how these terms are utilized by individuals is important for gaining an understanding of fungal pathogenesis mechanisms. A full discourse on the complicated history of these terms is not appropriate here and the reader is referred to recent articles discussing the history of “virulence” and “pathogenesis” for more in-depth analyses of these terms.<sup>13</sup> Here, we briefly discuss our preferred use of these terms, how they relate to human fungal pathogenesis, and why correct use of these terms is critical to our understanding of human fungal pathogenesis mechanisms.

The widespread and varied use of the terms “pathogenesis,” “virulence,” and “virulence factor” inhibits communication between scientists in different areas of microbial pathogenesis research. Importantly, it also prevents a true understanding of the biology and evolutionary history of these important pathogens, which has implications for affecting the research directions of the fungal pathogenesis community. Historically, the terms “virulence” and “pathogenesis” were developed based on the ability of microbes to cause disease. Subsequently, the use of these terms was pathogen-centric and implied that specific selection pressures from hosts led to virulence factor evolution. The term pathogenesis stems from the Greek *pathos* ‘disease’ and *genesis* ‘development’ and thus *pathogenesis*, simply stated, is the ability of an organism to cause disease. In contrast, the term *virulence* has been defined as the relative ability of a pathogen to cause disease. Hence, all fungal pathogens cause disease but some are more virulent than others, i.e., they have a greater ability to cause disease and damage to the host. This of course begs the question: Why are some fungal pathogens more virulent than others?

The answer to this question lies in the controversial term “virulence factor.” Virulence factors have been defined as components of a pathogen that permit a pathogen to cause disease. Typically, these components are genetically encoded

in the genome of the pathogen. Elimination of these components, through induced mutations in the laboratory or evolutionary mechanisms in the environment, reduces the virulence of the pathogen but not its viability. Thus, virulence factors have evolved specific functions required for pathogenesis. We consider this evolutionary component of the definition of a virulence factor a critical component in understanding microbial virulence.

Taking into account the likely evolutionary history of a gene or gene product allows the differentiation between a true virulence factor and a factor that has been called a virulence factor simply because in its absence the organism cannot cause disease. For example, genes essential for fungal growth *in vitro* have at times been termed virulence factors in the medical mycology literature. Yet, it seems clear that these genes have little to do with microbial virulence; rather, these genes have defined evolutionary functions involved in the basic biology of fungal organisms. If removal of this type of gene prevents or severely alters fungal growth and fitness *in vitro*, naturally these fungal strains will be incapable of causing disease *in vivo*.

Thus, if we accept the simple classic definition of virulence and virulence factor, most human fungal pathogens do not possess classic virulence factors. Yet when one begins to examine the literature on human fungal pathogenesis, it becomes difficult to ascertain what is or is not a virulence factor. This confusion stems from the requirement that for most human fungal infections to occur, deficiencies in the host immune system must be present. In other words, most human fungal pathogens cannot overcome an intact host immune system to cause disease. We, and many others, now argue that the use of the term “virulence factor” with regard to these important genes in normal fungal physiology and microbial fitness is confusing and fails to appreciate the biology of these important organisms. For this review, we would like to emphasize the evolutionary biology of these fungal pathogens, taking into consideration the potential and likely evolutionary mechanisms that led to the specific functions of genes and gene products. Thus, rather than taking the definitions of “virulence” and “virulence factor” and redefining them to fit new understandings of human fungal pathogenesis, we encourage the use of new terms that accurately reflect and appreciate the biology of these fungal pathogens as saprophytic organisms.

These terms should also accurately depict certain fungi’s unique ability to become pathogenic in specific conditions most often associated with immunosuppression of the host. We propose that human opportunistic fungal pathogens possess “virulence attributes” rather than “classic virulence factors.” Virulence attributes are components of these fungi that have arisen through selection pressures encountered throughout the course of evolutionary history to allow these fungi to adapt and complete their life cycles in their natural environments. Coincidentally, these attributes also allow certain fungi the ability to cause disease in immunocompromised hosts by allowing them to adapt and survive in an immunocompromised mammalian host, which quite often is an environment that is not unlike their natural ecological niches.

Next, we turn our discussion to the three main causative agents of human mycoses: *Aspergillus fumigatus*, *Cryptococcus neoformans*, and *Candida albicans*. In addition, we briefly discuss a fascinating attribute of a group of fungal pathogens termed the dimorphic fungi. In these discussions, we present



**Table 2-1** General characteristics of the three most commonly encountered fungal pathogens

Fungal pathogen	Ecologic niche	Primary morphology	Virulence attributes	Diseases
<i>Aspergillus fumigatus</i>	Soil, compost piles, organic debris	Conidia, filamentous hyphae	High temperature growth, oxidative stress resistance, fast growth rates, secondary metabolites	Invasive aspergillosis, allergic bronchopulmonary aspergillosis
<i>Cryptococcus neoformans</i>	Soil, trees, avian excreta	Yeast	High temperature growth, polysaccharide capsule, melanin production	Cryptococcal meningitis, pulmonary disease
<i>Candida albicans</i>	Human commensal	Yeast	High temperature growth, adherence, protease production, biofilm formation, dimorphism	Systemic, oral, and vaginal candidiasis

the main virulence attributes of these organisms (Table 2-1), their implications for human fungal infections, the probable evolutionary mechanisms by which these attributes have arisen, and possible novel therapeutic treatments that may be developed from this knowledge.

## ***Aspergillus fumigatus* – the menacing mould**

Advances in medical technologies have significantly increased our ability to treat patients with debilitating forms of cancer such as various forms of leukemia and whole organ failures. Currently, an estimated 15,000 allogeneic and 25,000 autologous stem cell transplants are performed worldwide yearly.<sup>14,15</sup> In addition, from 1998 to 2002, 113,682 solid organ transplants were performed in the United States, which is a 20% increase over the previous 5-year period.<sup>15,16</sup> Unfortunately, patients undergoing these life-saving procedures are at increased risk for infections by *Aspergillus fumigatus* and other *Aspergillus* species due to their immunocompromised condition. Overall, since 1980, the mortality due to *Aspergillus fumigatus* infections has increased 357%.<sup>12</sup> As medical technologies continue to advance and the immunocompromised patient population continues to increase, incidences of often fatal infections by *A. fumigatus* will continue to skyrocket. While recent studies with broad-spectrum azole prophylaxis in high-risk patient populations have been successful, *Aspergillus* infections have yet to be completely prevented from affecting specific patient populations.

Due to the urgent need to gain a better understanding of the pathogenesis mechanisms of this increasingly important filamentous fungus, molecular analyses of *A. fumigatus* pathogenesis mechanisms have also increased. The recent completion of a whole genome sequence for a clinical strain of *A. fumigatus* and other related *Aspergillus* species promises to quicken the pace of *A. fumigatus* pathogenesis research.<sup>17,18</sup> Currently, research on the molecular mechanisms of *A. fumigatus*

pathogenesis is underdeveloped. To date, no classic virulence factor has been identified in *A. fumigatus*; however, mutation of approximately 20 genes, all with roles in basic fungal biology, have resulted in reduced virulence in murine models of aspergillosis (Table 2-2).

Taken together, these results allow us to understand the unique virulence attributes that make *A. fumigatus* a lethal opportunistic pathogen. These attributes are thought to include thermotolerance, growth rates, conidium morphology, secondary metabolite production, degradative enzyme production, and oxidative stress resistance.<sup>19-22</sup> However, as indicated in Table 2-2, the majority of genes reported to be involved in *A. fumigatus* pathogenesis are directly related to primary metabolism and subsequently involved in fungal growth and development. Thus, research on *A. fumigatus* pathogenesis mechanisms to date strongly suggests that *A. fumigatus* does not possess virulence factors, but rather possesses a unique complement of virulence attributes for survival in immunocompromised mammalian hosts.

## **Growth and development**

Recently, it was hypothesized that the success of *A. fumigatus* as a pathogen was directly related to its increased in vitro growth rates.<sup>23</sup> Other support for this hypothesis has come from molecular analysis of genes involved in *A. fumigatus* growth and development. Steinbach et al<sup>24</sup> discovered that the calcium-calmodulin dependent serine/threonine protein phosphatase calcineurin was required for *A. fumigatus* growth and development. A strain of *A. fumigatus* with the catalytic domain of calcineurin replaced by a selectable marker displayed severe growth defects, including lack of polarized growth and morphologic defects related to conidium development and morphology (Fig. 2-1). Not surprisingly, this calcineurin mutant was unable to establish disease in various murine models of invasive aspergillosis, confirming that inhibition of *A. fumigatus* hyphal growth and development in vitro correlates with the inability to cause disease. These results were also

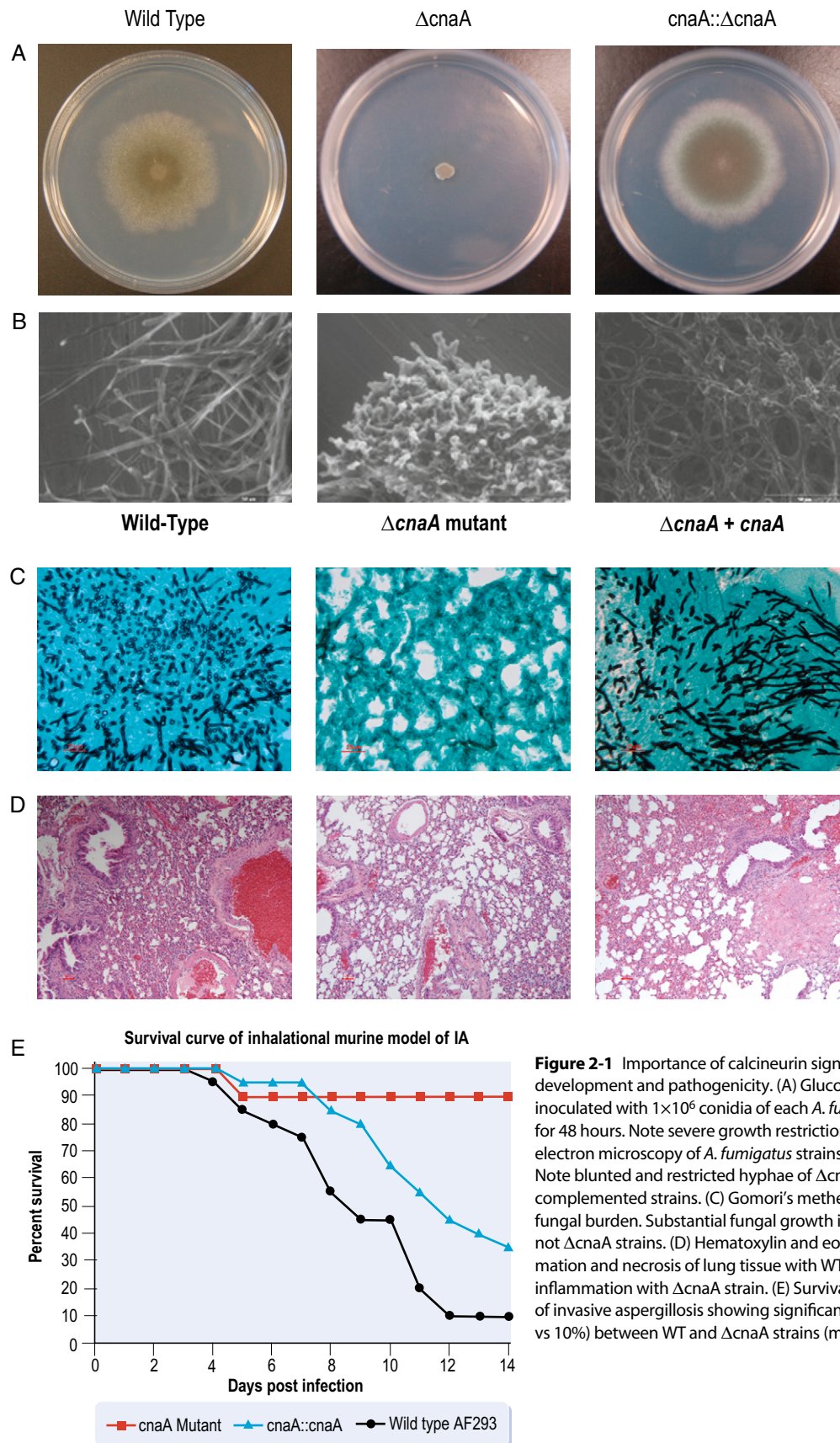
**Table 2-2** Genes reported to be involved in *Aspergillus fumigatus* pathogenesis

Gene	Cellular function	Animal model	Reference
pyrG	Pyrimidine biosynthesis	Murine intranasal	155
chsG	Chitin biosynthesis	Murine intranasal	156
areA	Transcriptional regulator – nitrogen metabolism		157
pksP, alb1	DHN-melanin biosynthesis	Murine intravenous	62, 158
pabaA	Folate biosynthesis	Murine intravenous and intranasal	159, 160
fos1	Histidine kinase two-component stress response signaling	Murine intravenous	161
cat1 and cat2	Oxidative stress response	Rat intratracheal	162
rhbA	Nitrogen sensing	Murine intranasal	163
cgrA	Ribosome biosynthesis	Murine intranasal and <i>Drosophila</i> model	51
sidA	Siderophore biosynthesis		49
cpcA	Transcriptional regulator – amino acid biosynthesis	Murine intranasal	27
lysF	Lysine biosynthesis	Murine intranasal	164
gpaB	cAMP signaling	Murine intranasal	59
pkaC1	cAMP signaling	Murine intranasal	59
acyA	cAMP signaling	Murine intranasal	58, 59
laeA	Transcriptional regulator – secondary metabolism	Murine intranasal	35, 36
rasB	Polarized hyphal growth	Murine intranasal	165
pkaR	Growth, morphology and oxidative stress resistance, cAMP signaling	Murine intranasal	61
gel2	GPI-anchor protein, $\beta(1-3)$ glucanosyltransferase	Murine intranasal	166
cnaA	Polarized hyphal growth, hyphal and conidial morphology	Murine inhalational, intranasal and intravenous	24
pes1	Oxidative stress, conidial morphology	Galleria moth model	167
ppoA, B, and C	Prostaglandin production	Murine intranasal	43

confirmed by Ferreira et al<sup>25</sup> with a calcineurin mutant in another strain of *A. fumigatus* and with a separate aspergillosis animal model.

Molecular analyses of the signaling pathways mediated by calcineurin in *A. fumigatus* are currently under way. However, these preliminary results may shed light on the potential of genes involved in fungal growth and development to be used as antifungal targets. Two well-studied calcineurin inhibitors

currently exist: cyclosporin A and FK506 (tacrolimus). These inhibitors bind to the immunophilins cyclophilin and FKBP12, respectively, and consequently the immunophilin–drug complexes inhibit calcineurin function.<sup>26</sup> Thus, addition of these drugs to current antifungal drug therapies may improve aspergillosis patient outcomes. However, given the highly conserved nature of the calcineurin signaling pathway (it is conserved in other higher eukaryotes including humans), it may



**Figure 2-1** Importance of calcineurin signaling in *Aspergillus fumigatus* growth, development and pathogenicity. (A) Glucose minimal medium plates were inoculated with  $1 \times 10^6$  conidia of each *A. fumigatus* strain and incubated at  $37^\circ\text{C}$  for 48 hours. Note severe growth restriction of  $\Delta cnaA$  mutant. (B) Scanning electron microscopy of *A. fumigatus* strains grown in GMM broth for 48 hours. Note blunted and restricted hyphae of  $\Delta cnaA$  mutant compared to WT and complemented strains. (C) Gomori's methenamine silver staining showing fungal burden. Substantial fungal growth is seen in WT and complemented but not  $\Delta cnaA$  strains. (D) Hematoxylin and eosin staining showing massive inflammation and necrosis of lung tissue with WT and complemented strains, and mild inflammation with  $\Delta cnaA$  strain. (E) Survival curve of inhalational murine model of invasive aspergillosis showing significant ( $P < 0.001$ ) decrease in mortality (90% vs 10%) between WT and  $\Delta cnaA$  strains (modified from Steinbach et al.<sup>24</sup>).

be difficult to selectively target the calcineurin pathway in the fungal pathogen without adversely affecting the corresponding signaling pathway in the host. Inhibition of calcineurin signaling in humans leads to increased immunosuppression, which could further exacerbate fungal infections. Further dissection of the *A. fumigatus* calcineurin signaling pathway may reveal fungal-specific targets that may be more effective antifungal targets than calcineurin itself.

Another highly conserved signaling pathway recently identified to be important in *A. fumigatus* pathogenesis is the cross-pathway control system, which modulates fungal amino acid biosynthesis. Krappmann et al<sup>27</sup> identified an ortholog of the yeast transcriptional activator protein, Gcn4p, in *A. fumigatus* and named it *cpcA*. Mutants with a deleted *cpcA* locus are hypersensitive to the tryptophan analog 5-methyltryptophan, which induces a starvation response and decreased growth rates in the mutant strains. However, *cpcA* mutants ultimately displayed no specific nutritional requirements and therefore are prototrophic for amino acids, purines or pyrimidines. Interestingly, in a neutropenic intranasal model of invasive aspergillosis, *cpcA* mutant strains displayed an approximate 50% reduction in virulence (measured by mortality). The authors hypothesize that this reduction in virulence might be attributable to imbalances in the amino acid pool available in vivo or that *cpcA* is required for the transcription of an unidentified *A. fumigatus* virulence factor. Further studies are needed to elucidate the exact role of *cpcA* in *A. fumigatus*, and these studies have the potential to reveal important information about the environment encountered by *A. fumigatus* in a mammalian lung. However, the apparent involvement of the *A. fumigatus* cross-pathway control system in pathogenesis further suggests that mechanisms of saprophytic growth and microbial fitness are also required for fungal pathogenesis.

## Secondary metabolism

Many fungi, including *Aspergillus* species, produce a diverse array of biologically active small molecular weight metabolites. Collectively these metabolites have been termed secondary metabolites, and include many well-known pharmaceutical agents such as cyclosporin and penicillin.<sup>28</sup> In several plant-fungal interactions, secondary metabolites have been found to be the primary virulence factor of the fungus.<sup>29,30</sup> *Aspergillus fumigatus* is known to produce several biologically active metabolites that are known to be immunosuppressive, and thus secondary metabolite production has been hypothesized to be an important virulence attribute of *A. fumigatus*.<sup>31,32</sup> Recent phylogenetic and genomic analyses of *Aspergillus* whole genome sequences have revealed substantial diversity in the ability to produce secondary metabolites.<sup>18,33,34</sup>

Support for the potential role of secondary metabolites in *A. fumigatus* pathogenesis can be seen with strains lacking the transcriptional regulator *laeA*. Mutant *laeA* strains are deficient or have significant reductions in the production of gliotoxin, helvolic acid, fumagillin, and other unknown secondary metabolites.<sup>35,36</sup> Sequence analysis of the *laeA* protein showed no definitive similarity to known proteins, but suggested that *laeA* might be a protein methyltransferase involved in altering chromatin structure.<sup>37,38</sup> Of significance, an *A. fumigatus* strain lacking *laeA* was found to be avirulent in a murine intranasal model of invasive aspergillosis. This result suggests a potential

role for secondary metabolites in *A. fumigatus* pathogenesis; however, the pleiotropic nature of *laeA* loss prevents this conclusion from being definitive. The authors, however, hypothesized that significant reduction in gliotoxin production in the *laeA* mutant may account for the avirulent phenotype due to gliotoxin's known immunosuppressive properties.

The direct role of gliotoxin was subsequently examined by creating a mutant in the non-ribosomal peptide synthetase responsible for gliotoxin production, *gliP*.<sup>39,40</sup> Two independent labs with different *Aspergillus* background strains and animal models produced similar results regarding the *gliP* mutant phenotype. *gliP* mutant strains displayed normal growth and morphology in vitro, and concomitantly were capable of causing similar mortality compared with their respective wild-type strains in distinct murine models of invasive aspergillosis. These results suggest that gliotoxin is not required for disease development in these murine models.

However, gliotoxin is a strong immunosuppressant and in these animal models, as in the majority of patients with aspergillosis, severe immunosuppression is already present prior to the fungal infection. Thus, the additional immunosuppressive activity of gliotoxin may not be critical for disease establishment. However, these results do not rule out a role for gliotoxin in chronic forms of aspergillosis such as allergic bronchopulmonary aspergillosis, or even in making subtle differences in host immune system responses that could affect patient outcomes depending on the pathophysiology of the underlying immune system defect. Indeed, culture filtrates from *gliP* mutant strains lacking gliotoxin were unable to inhibit ionomycin-mediated mast cell degranulation, which may suggest that gliotoxin could play a role in immunomodulation in chronic forms of aspergillosis.<sup>39</sup> Further, it could significantly alter the ability of the immune system to reconstitute after bone marrow or solid organ transplants. Thus, the exact role of gliotoxin production, if any, in aspergillosis is still not fully understood.

Another mechanism of host immune system modulation by *A. fumigatus* and related *Aspergillus* species may be the production of prostaglandins. Prostaglandins are eicosanoids and comprise a subclass of C<sub>20</sub> oxylipins. Eicosanoids are known to be involved in numerous immune system activities including regulation of inflammation, pain, and allergic responses.<sup>41</sup> It has been hypothesized that production of eicosanoids and other oxylipins could be manipulated to treat fungal infections.<sup>42</sup> RNA silencing was used to silence three cyclooxygenase genes (*ppoA*, *ppoB*, *ppoC*) in *A. fumigatus* that were predicted to produce prostaglandins.<sup>43</sup> Silencing led to loss of prostaglandin detection in fungal culture filtrates and, most interestingly, hypervirulence in an intranasal murine model of invasive aspergillosis. Additional in-depth animal model experiments are currently needed to examine this interesting and potentially important finding that implies host-fungal communication via the production of small molecules.

The host environment clearly plays an important role in determining the outcome of microbial infections.<sup>44</sup> One nutrient required by both pathogenic bacteria and fungi that is not readily available in the human body is iron. Both bacteria and fungi produce low molecular weight metabolites called siderophores, that bind Fe(III) with high affinity.<sup>45-47</sup> The role of iron in bacterial pathogenesis has been extensively explored and confirmed, and recently the production of siderophores by *A. fumigatus* has been shown to be critical for *Aspergillus*



infections.<sup>48,49</sup> *Aspergillus fumigatus* is able to grow in iron-poor human serum, and it was hypothesized that this survival was due to siderophore production.

Confirmation of this hypothesis was obtained by creation of an *A. fumigatus* strain deficient in the siderophore biosynthetic gene, *sidA*. *sidA* encodes a L-ornithine (N5oxygenase) involved in the first step of hydroxamate siderophore biosynthesis. Deletion of *sidA* resulted in significant fungal growth defects in culture medium and serum with low iron concentrations. The *sidA* mutants were found to lack production of triacetylfusarine C and ferricrocin siderophores. Importantly, the *sidA* mutant strains were completely avirulent in an intranasal murine model of invasive aspergillosis. In fact, sparse or no fungal hyphae were found in lung tissue samples from *sidA* mutant-infected mice, indicating that *sidA* mutants could not grow in vivo.<sup>49</sup> Thus, the ability to scavenge iron in the lungs of immunocompromised hosts appears to be a critical virulence attribute for fitness of *A. fumigatus* in vivo. Hematologic malignancy patient populations at high risk for invasive aspergillosis often receive many blood transfusions providing exogenous iron, and thus development of a treatment strategy that inhibited the ability of *A. fumigatus* to sequester iron in vivo may potentially improve treatment outcomes.

## Thermotolerance

The ability of *A. fumigatus* to survive temperatures up to 70°C is a unique attribute in the *Aspergillus* genus and fungal kingdom in general. This thermotolerant phenotype has been hypothesized to be an important virulence attribute of *A. fumigatus*.<sup>19</sup> Molecular analysis of genes involved in thermotolerant growth has recently shed light on this hypothesis. A gene required for growth at 42°C was recently identified by complementation of a mutant unable to grow at this temperature. The gene, *THTA*, has an unknown function, but deletion of this gene in a wild-type strain of *A. fumigatus* confirmed its role in thermotolerant growth.<sup>50</sup> However, virulence of the *THTA* mutant strain was unaffected in a murine model of invasive aspergillosis, indicating that growth at physiologic temperatures was not affected by loss of *THTA*. Thus, while growth at physiologic temperatures is clearly required to cause disease, thermotolerant growth may not be an important virulence attribute of this organism.

The importance of growth at physiologic temperatures was confirmed by deletion of a gene involved in ribosome biogenesis. Deletion of this gene, *cgrA*, results in delayed germination and reduced growth rates at 37°C.<sup>51</sup> The *cgrA* mutant has significant decreases in mortality in a murine model of invasive aspergillosis and in a *Drosophila* (fruit-fly) Toll receptor-deficient model. The decrease in virulence in the *Drosophila* model, which is conducted at 25°C, also points to the importance of timely conidial germination in *A. fumigatus* pathogenesis. Clearly, genes that are required for fungal survival and/or optimal growth rates at physiologic temperatures are excellent targets for antifungal drug development.

## Oxidative stress

It is likely that most mammalian microbial pathogens encounter oxidative stress during pathogenesis and *A. fumigatus* is no exception. Inhaled fungal conidia are engulfed and attacked

by alveolar macrophages, which generate reactive oxygen species (ROS) that can kill the fungal conidia.<sup>52,53</sup> Fungal hyphae growing in mammalian hosts are attacked by neutrophils which utilize ROS to kill the invading fungus.<sup>54-56</sup> For example, the human condition chronic granulomatous disease (CGD) is characterized by the inability of immune effector cells to generate an oxidative burst.<sup>57</sup> Interestingly, in CGD patients who develop aspergillosis, typically patients acquire more *A. nidulans* than *A. fumigatus* infections, possibly suggesting that the ability to tolerate oxidative stress is greater in *A. fumigatus* than *A. nidulans*.

Recent molecular analysis of genes involved in oxidative stress tolerance in *A. fumigatus* has clearly shown the importance of this attribute in fungal pathogenesis. For instance, genes involved in cyclic AMP-dependent signaling pathways were found to be essential for *A. fumigatus* pathogenesis.<sup>58-61</sup> Conidia of deletion mutants in adenylate cyclase (*acyA*) and the G-protein alpha subunit (*gpaB*) were more sensitive to killing by human monocyte-derived macrophages.<sup>58</sup> One possible explanation for these results is that cAMP signaling was found to, in part, regulate expression of *pksP*, a gene encoding a polyketide synthetase involved in melanin production. *pksP* mutants have white conidia compared to the greenish-gray pigment of wild-type conidia, and are more susceptible to ROS.<sup>62</sup> Fungal melanins are proposed to protect fungi from ROS, ultraviolet light, and enzyme stresses. Thus regulation of melanin production by cAMP-dependent signaling may be required for fungal pathogenesis.<sup>63</sup> However, melanin production is also found widely in non-pathogenic saprophytic fungi, and the functions that melanin performs in vivo during infections are likely required during saprophytic growth in complex microbiologic communities. The cAMP signaling cascade illustrates the point that many of these types of regulatory gene networks and resulting phenotypes work in concert to establish disease. Whether the fungus is in an immunocompromised host or a compost pile, it is using multiple complex genetic interactions to survive the harsh environments.

## *Aspergillus fumigatus* virulence attributes – where did they come from?

An examination of the *A. fumigatus* virulence attributes and the genes that are responsible for these attributes begs the following questions. Did these attributes arise during the course of evolutionary history as the result of selective pressures from interactions with mammalian hosts (and hence are virulence factors in the classic sense)? Or did these virulence attributes arise from other environmental selective pressures that have little to do with fungal virulence, yet in combination, coincidentally allow *A. fumigatus* to infect immunocompromised hosts? While direct studies addressing these questions have yet to be undertaken with *A. fumigatus*, an argument can be made that none of the genes listed in Table 2-2 are classic virulence factors. Instead, an explanation of the occurrence of these genes can be found from likely selective pressures encountered by *A. fumigatus* in its natural ecologic niche, the soil. Ultimately selective pressures from this environment, particularly in compost piles where *A. fumigatus* is frequently found, resulted in a unique combination of attributes that coincidentally also allow *A. fumigatus* to thrive in immunocompromised mammals. Possible sources of these selective pressures include:

heat stress in compost piles, competition from other microorganisms for limited nutrient supplies, predation by soil nematodes and protozoa, and ultraviolet radiation exposure. One can envision how these environmental pressures could lead to development and persistence of many, if not all, of the *A. fumigatus* virulence attributes identified to date.

Recently, questions regarding the evolution of human fungal pathogenesis have begun to be experimentally examined in the yeast fungal pathogen *Cryptococcus neoformans*, and we now turn our discussion to this sugar-coated killer.

## ***Cryptococcus neoformans* – the sugar-coated killer**

Like *Aspergillus* species, *Cryptococcus* species are found abundantly in the environment often associated with soil, trees, and avian excreta.<sup>64</sup> Unlike the *Aspergilli*, *Cryptococcus* species are basidiomycete yeasts, giving them morphology distinctly different from hyphae of filamentous fungi like *A. fumigatus*. Infections caused by *Cryptococcus* species have concomitantly risen with the worldwide increase in immunocompromised patient populations in the last two decades, and have been particularly associated with the AIDS epidemic. The best estimates of *Cryptococcus* infections pre-AIDS epidemic predicted 0.8 cases per million persons per year.<sup>65</sup> During the peak of the AIDS epidemic within the United States, incidences of *Cryptococcosis* dramatically increased to five cases per 100,000 persons in urban areas, and in less developed countries afflicted with the AIDS epidemics, prevalence rates of 15–45% in AIDS patients have been reported.<sup>65,66</sup>

Recently, an outbreak of cryptococcosis occurred on Vancouver Island, British Columbia, Canada. Of particular interest to our discussion of fungal pathogenesis, afflicted patients and animals demonstrated no detectable abnormal immune system functions, indicating that this strain of *Cryptococcus* (now a separate species, *C. gattii*, formerly *C. neoformans* var. *gattii*), is capable of causing disease in immunocompetent hosts, and hence contains classic virulence factors.<sup>67</sup> This finding has direct implications regarding the generally accepted belief that opportunistic fungal pathogens have not evolved classic virulence factors, and will be discussed later in this section.

In order to combat this important emerging fungal pathogen, substantial efforts have been made to develop molecular tools to explore mechanisms of *Cryptococcus* pathogenesis. Unlike *A. fumigatus*, a defined sexual cycle for *C. neoformans* is available to conduct genetic studies and furthermore, the molecular manipulation of the fungus is relatively straightforward and successful compared to *A. fumigatus*. Several genome sequences are currently available or on the way to completion for the various *C. neoformans* strains and “new” species, *C. gattii*.<sup>68</sup> Consequently, substantial progress has been made in elucidating the virulence attributes of *Cryptococcus* species at the molecular level, and some now consider *C. neoformans* a model organism for the study of human fungal pathogenesis.<sup>64</sup>

Studies on the pathogenesis mechanisms of *C. neoformans* have revealed three essential virulence attributes: high temperature (37–39°C) growth, formation of an extracellular polysaccharide capsule, and synthesis of melanin (Fig. 2-2).<sup>66,69,70</sup> A substantial number of genes and complex regulatory pathways have been shown to mechanistically contribute to these

three major components of *C. neoformans*’ ability to cause disease (for a list of these genes, see Perfect<sup>71</sup>). In addition, several excellent reviews focusing on these well-established aspects of *Cryptococcus* pathogenesis are available.<sup>64,66,69,71-77</sup> For our discussion of mammalian fungal pathogenesis mechanisms, we will focus on recent studies utilizing *Cryptococcus* to understand the evolution and potential sources of human fungal pathogenesis.

## **Alternative hosts as reservoirs for selective pressure that led to virulence attribute development and persistence**

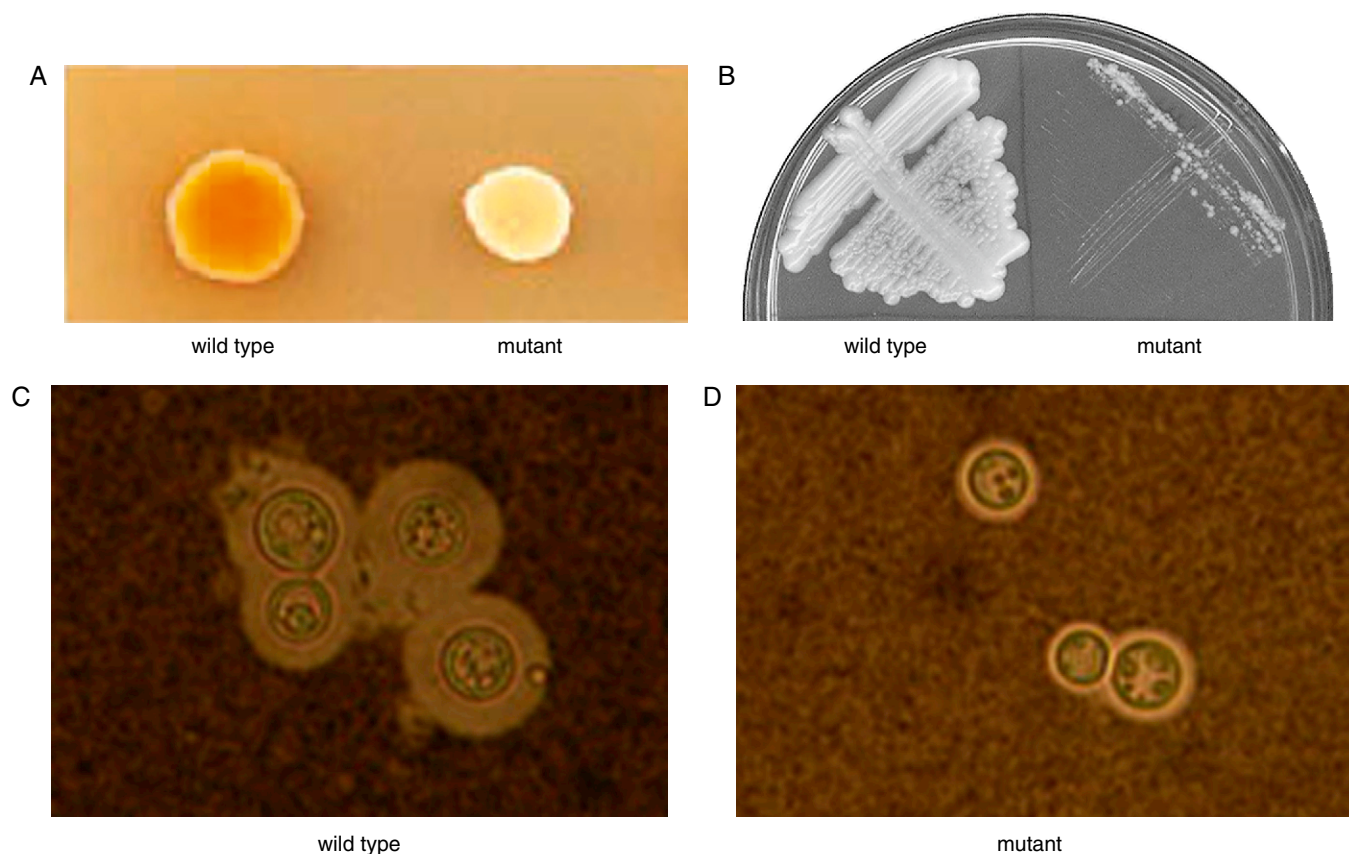
While the mechanisms and persistence of virulence in fungal pathogens of plants are generally well accepted, we still do not understand the same phenomenon in human fungal pathogens. It is clear that certain fungi possess what others and we term virulence attributes or a virulence composite, which allow these fungi to cause disease in mammals. Recently, evidence has been gathered that suggests that fungal interactions with other microorganisms in the environment could explain, in part, the evolution and persistence of mammalian fungal pathogenesis mechanisms.

In many ways, *C. neoformans* is a unique and ideal model to explore this hypothesis. Unlike *A. fumigatus*, *C. neoformans* infections can be latent and persist inside macrophages in immunocompetent individuals.<sup>78</sup> The polysaccharide capsule is primarily responsible for *C. neoformans*’ ability to persist in macrophages, and when host immune defenses deteriorate, shedding of capsule into macrophage vacuoles induces host cell cytotoxicity.<sup>79</sup> Thus, the question arises: how has *C. neoformans* gained the ability to persist and replicate inside macrophages when it is not required to complete its life cycle? To address this question, Steenbergen et al<sup>80</sup> built on earlier observations that *C. neoformans* is readily phagocytosed by soil-dwelling protozoa.<sup>81</sup> Interestingly, amoebae and macrophages share many of the same biologic properties including the ability to phagocytose particles, sequester particles in vacuoles, and secrete lysosomal enzymes to digest the engulfed particles.<sup>82</sup>

Steenbergen and colleagues hypothesized that interactions with soil-dwelling protozoa led to the ability of *C. neoformans* to become an intracellular facultative pathogen.<sup>80</sup> It was found that the soil-dwelling amoeba *Acanthamoeba castellanii* readily engulfed *C. neoformans* cells. Importantly, wild-type *C. neoformans* were able to replicate within the amoebae, which resulted in increased numbers of polysaccharide-containing vesicles that eventually caused amoebae cell death. However, a *C. neoformans* mutant strain defective in capsule production was unable to survive phagocytosis by amoebae. This acapsular strain also was protected from amoebae killing by melanization. Interestingly, the non-pathogenic common baker’s yeast *Saccharomyces cerevisiae* and the human commensal opportunistic pathogen *Candida albicans* were unable to survive phagocytosis by *A. castellanii*.

These results support the hypothesis that virulence attributes of *C. neoformans* such as capsule production and melanization may have arisen through selection pressures resulting from environmental interactions with soil-dwelling amoebae. This hypothesis has been further supported by studies demonstrating that virulence attributes of *C. neoformans* are also required for pathogenesis of the model organisms *Caenorhabditis elegans*,





**Figure 2-2** Virulence attributes of *Cryptococcus neoformans*. (A) Melanin formation by *C. neoformans*. Lack of melanin production is seen in  $\Delta gpa1$  strain, which is also attenuated for virulence in a murine model of cryptococcosis (modified with permission from reference 168). (B) High temperature growth of *C. neoformans*. Lack of growth at 37°C is seen with a  $\Delta ras1$  strain (modified from reference 169). (C and D) Capsule formation by *C. neoformans*. Significant decrease in capsule formation is seen with a  $\Delta nrp1$  strain (photo courtesy of Dr Connie Nichols, Duke University Medical Center).

a soil-dwelling nematode known to ingest bacteria and yeast for food, and *Dictyostelium discoideum*, a free-living soil amoeba often used as a model for the study of phagocytic processes.<sup>83-85</sup> *Cryptococcus neoformans* strains grown in the presence of *D. discoideum* were hypervirulent compared to *C. neoformans* strains grown alone. In vitro characterization of the hypervirulent strain revealed increased capsule production and melanization, strongly suggesting that interactions with soil-dwelling organisms can rapidly alter the virulence of *C. neoformans*.<sup>85</sup>

This apparent phenotypic plasticity of *C. neoformans* to alter properties in response to environmental conditions may have direct relevance to the development of mammalian fungal virulence in this genus. For example, the recent outbreak of cryptococcosis on Vancouver Island in Canada occurred in apparently immunocompetent patients and animals.<sup>67,86</sup> Molecular analyses of the Vancouver Island *C. gattii* isolates revealed the primary population to be clonal and of the alpha mating type with a molecular type of VGII.<sup>87</sup> It appeared that two strains mated and the resulting recombinant strain was more virulent in animals similar to the strain that causes over 90% of the reported infections in the outbreak.<sup>88</sup> The question becomes: how and when did this strain of *C. gattii* evolve the ability to infect immunocompetent hosts? An alternative hypothesis, however, is that there is something unique about the immune system of afflicted individuals not yet detected that allowed these strains to cause disease.

However, as seen with the *Dictyostelium* experiments, *Cryptococcus* species can rapidly respond to changing environments and alter their virulence or phenotypically switch colony morphology. While the mechanisms for this phenotypic plasticity are currently unknown, it has been hypothesized that a recent genetic recombination event in an unusual fertile clade of *C. gattii* with increased basidioconidium production and/or altered environmental niche is responsible for the Vancouver Island outbreak.<sup>89</sup> Thus, environmental selection pressures may increase the virulence of *Cryptococcus* species. These selection pressures may be driven by yeast interactions and response to soil microorganisms and result in the persistence of virulence attributes in selected populations, increasing encounters with immunocompromised hosts, and the ability to create virulence diversity in the yeast population through sexual recombination.<sup>90</sup> Future mechanistic and population genetic studies will undoubtedly continue to shed light on the evolution of mammalian fungal virulence attributes and *Cryptococcus* species are ideal for these studies.

## *Candida albicans*

From thrush to vaginal infections to candidemia, infections caused by *Candida* species are likely the fungal infections most familiar to clinicians and patients. In the United States,

*Candida* infections are the fourth most common cause of nosocomial infections.<sup>91-93</sup> The most common species identified in human infections is *C. albicans*; however, additional species are increasing in frequency and include: *C. dubliniensis*, *C. glabrata*, *C. krusei*, *C. lusitanae*, *C. parapsilosis*, and *C. tropicalis*.<sup>94,95</sup> *C. albicans* can be isolated from the oropharynx of over 40% of normal individuals and is a standard commensal of the lower gastrointestinal tract.<sup>96</sup>

Like *Aspergillus* and *Cryptococcus*, the vast majority of candidiasis cases occur in immunocompromised individuals. However, *Candida* infections are unique because they typically are acquired endogenously. Unlike *Aspergillus* and *Cryptococcus* species, *C. albicans* is seldom found in the soil or external environment.<sup>97</sup> Instead, it is a commensal and normal inhabitant of the human microflora. This feature of *C. albicans* biology has significant ramifications for our discussion of fungal pathogenesis, and it is likely that the key attributes which allow it to be a commensal will integrally be used in its pathogenic fitness profile.

Previously, we discussed the interplay between selection pressures in the soil environment and the evolution of virulence attributes in *A. fumigatus* and *C. neoformans*. Yet *C. albicans* is already highly adapted to the host environment since it is a common commensal of mucosal surfaces in mammals, especially humans. Some of these adaptations necessary for growth and persistence in humans include: adherence to mucosal surfaces, ability to withstand normal and fever-induced body temperature (37–39°C), and the innate ability to rapidly adapt to changing microenvironments in the host. In order to persist as a commensal, *Candida* must have evolved elaborate mechanisms to evade or minimize host immune responses without causing disease. In addition, because *C. albicans* can colonize a diverse array of host environments (oral, vaginal, gastrointestinal), it must also possess the ability to survive in these disparate host environments. Below, we discuss some of the unique aspects of *C. albicans* biology and their impact on fungal pathogenesis.

## Adherence to host tissue

Adherence to host tissue is required for commensals, like *Candida*, to colonize hosts and cause disease. Recent studies utilizing *C. albicans* have identified many of the fungal adhesins that this fungus uses to adhere to various host tissues.<sup>98-100</sup> One gene family in particular, the agglutinin-like sequence (ALS) gene family, has received extensive attention. The ALS gene family contains at least eight members that are characterized by the presence of conserved tandem repeats in the central region of the proteins.<sup>101</sup> The C-terminus of the proteins contains a glycosylphosphatidylinositol (GPI) anchor site that anchors the proteins to the fungal cell wall (Fig. 2-3).

Substrate binding studies using both gene knockouts of the ALS gene family members as well as heterologous expression in the model non-pathogenic yeast *Saccharomyces cerevisiae* have identified the potential binding specificities of the ALS gene family members.<sup>98,102-107</sup> *Als1* and *Als3* null mutants have reduced adherence to endothelial cells and overexpression of these two ALS gene family members in *S. cerevisiae* increases adherence. *Als3* null mutant also had reduced adherence to oral epithelial cells. Expression of *Als1*, *Als3*, and *Als5* in *S. cerevisiae* increased adherence to a broad range of substrates

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**Figure 2-3** Conceptual model of structural-functional relationships in Als family proteins. Als proteins are composed of three general components: an N-terminal domain, serine/threonine-rich tandem repeats, and a serine/threonine-rich C-terminal domain containing a glycosylphosphatidylinositol anchor that is bound to the *C. albicans* cell wall. As illustrated, Als proteins contain multiple conserved anti-parallel beta-sheet regions (CR1-n) that are interposed by extended spans, characteristic of the immunoglobulin superfamily. Projecting from the beta-sheet domains are loop/coil structures containing the hypervariable regions (HVRs). The three-dimensional physicochemical properties of specific Als protein HVRs probably govern interactions with host substrates that confer adhesive and invasive functions to *C. albicans*. For illustrative purposes, only three N-terminal beta-sheet/coil domains and their respective CR/HVR components are shown (reproduced with permission from Filler et al.<sup>170</sup>).

including collagen, endothelial cells, fibronectin, laminin, and oral epithelial cells.<sup>107</sup> In addition, *Als3* was found to mediate endocytosis by endothelial cells, and thus *Als3* is likely a critical factor involved in endothelial cell invasion.

Of interest to fungal pathogenesis mechanisms is the lack of similarity between adhesins in *Candida* and other fungal pathogens. Thus, the ability to adhere to substrates is likely specific for the ecologic niche that each fungus typically occupies. The human commensal *C. albicans* is uniquely positioned to adhere to host tissues, unlike *A. fumigatus* or *C. neoformans* that are typically found in the soil and are not persistent mammalian colonizers. One could hypothesize that *A. fumigatus* and *C. neoformans* possess adhesins that allow them to adhere to important substrates in their natural environments such as plant and other organic debris. However, it is clear that even *A. fumigatus* and *C. neoformans* adhere to host tissues prior to their invasion, indicating that their respective adhesins may have overlapping substrate specificity. Future studies will undoubtedly discover the specific adhesive proteins and molecules in these important fungal pathogens and may be used to compare and contrast with *Candida* adhesins.

In relation to adherence to host tissue, *C. albicans* and other pathogenic *Candida* species also produce secreted aspartic proteinases (SAP) that have long been known to be important for interactions with host cell surfaces and for virulence.<sup>108</sup> SAPs have been shown to degrade host cell surface molecules and degrade tissue, allowing *Candida* infections to disseminate. Recently, it was shown that SAPs could alter the host immune

response via modulation of epithelial cytokine response in an in vitro model of vaginal candidiasis.<sup>109</sup> Ultimately, SAPs may be critical factors in altering host cell surfaces, affecting immune system responses and also allowing greater adherence and tissue invasion of *Candida* cells.

## Biofilm formation

It is now clear that many microorganisms grow in complex communities on specific substrates in their ecologic environments, rather than as single free-living organisms.<sup>110,111</sup> These substrate-attached communities are frequently referred to as biofilms characterized by a secreted extracellular matrix around a colony of cells, and it has become clear that biofilm formation has a significant impact on human health.<sup>111-115</sup> Importantly, *C. albicans* can form extensive biofilms on medically implanted, indwelling devices such as catheters.<sup>116</sup> Since *C. albicans* is a human commensal, it can frequently come into contact with indwelling medical devices, attach, develop a biofilm, and cause severe infections. In fact, a substantial number of *C. albicans* infections are now reported to be associated with biofilm formation.<sup>116,117</sup> This has direct effects on clinical management of these kinds of *Candida* infections as antifungal activity in biofilms is altered compared to free-living yeast cells.

The importance of biofilm formation in *C. albicans* infections has resulted in focused research aimed at elucidating the molecular mechanism(s) of biofilm formation. Biofilm formation is a complex developmental and genetically controlled phenomenon with three basic stages (reviewed by Nobile and Mitchell<sup>118</sup>):

- attachment and yeast cell colonization of substrate
- yeast cell growth and proliferation forming a basal layer of yeast cells
- pseudohyphae, hyphal extension, and concomitant production of an extracellular matrix.

Recent data suggest that *C. albicans* cells can detect the presence of a specific surface and in response initiate a transcriptional program leading to biofilm formation. For example, within 30 minutes of contacting a polystyrene surface, yeast cells initiate a distinct transcript profile apparently directly related to surface contact.<sup>119</sup> Of potential clinical interest, azole efflux pump genes, *CDR1* and *MDR1*, are transcriptionally induced within 6 hours of surface contact.<sup>120</sup> This surface contact induction of efflux pump genes may help explain the significant increase in azole resistance found in biofilm cells.<sup>121</sup> Biofilm drug resistance may also be due to the morphologic heterogeneity of biofilms. Biofilms contain all three forms of *C. albicans* including yeast cell, pseudohyphae, and hyphae. For instance, it has been reported that pseudohyphae and hyphae are more resistant than yeast cells to the biocide chlorhexidine.<sup>122</sup> It has also been shown that yeast in biofilms are resistant to azoles and standard polyenes but there is antifungal activity with the echinocandins and lipid products of amphotericin B.<sup>123</sup>

Interestingly, recent results have shown that biofilm formation is inhibited by alcohol dehydrogenase activity via an ethanol-dependent mechanism.<sup>124</sup> This may suggest that a novel treatment strategy using ethanol for the elimination of *Candida* biofilm formation could be developed.

## Adaptation to host environment

One environmental characteristic of mammals is the diverse pH range of various tissues in the body. Consequently, *C. albicans* must be able to adapt and respond to a wide range of pH conditions during colonization of distinct host tissues. For example, it must be able to respond to the changing pH of the vaginal tract dependent upon the menstrual cycle of the host. The ability of *Candida* to respond to these diverse extracellular pH changes is governed by a conserved network of genes regulated in part by a zinc finger transcription factor *RIM101/PacC*.<sup>125-128</sup> Specifically, this pathway allows *Candida* to respond to neutral and alkaline pH typically found in mammalian hosts. Using mutants of the *RIM101* pathway, it has been shown that this ability to respond to environmental pH is required for systemic candidiasis.<sup>128</sup> Perhaps importantly, environmental pH has also been found to be a potent regulator of the yeast to hyphal transition often associated with *Candida* pathogenesis. Mutants in several of the *RIM101* pathway components, including *RIM101* itself, fail to form hyphae at pH 8 and remain as yeast.<sup>127</sup>

## Dimorphism

*Candida albicans* is a dimorphic fungus; it can exist as yeast cells, pseudohyphae or hyphae. This ability to change morphology has been hypothesized to be an important virulence factor of *C. albicans*. The hypothesis generally states that hyphal forms of the fungus are invasive while the yeast morphology is non-invasive. Several studies have suggested a correlation between morphology and virulence in murine models.<sup>129,130</sup> Yet the pleiotropic nature of the mutations in the strains utilized in these studies make the conclusion that morphology is directly linked to virulence untenable.<sup>131</sup> In fact, in studies with mutants of the gene *NRG1* (negative regulator of filamentation) driven by a tetracycline-inducible promoter, there was no correlation between fungal burden, mortality, and hyphal mortality in a murine model of candidiasis.<sup>130</sup> Furthermore, *C. glabrata* does not have the ability to produce hyphae and is a prominent opportunistic pathogen. Perhaps more likely is the hypothesis that in vivo fitness of *C. albicans* is directly linked to the unique host microenvironments that it encounters during pathogenesis.<sup>132</sup> Thus, certain morphologies of the fungus may be more critical than others, depending on the location of the infection within the host.

On the other hand, dimorphism has been definitively shown to be a critical virulence attribute in a small group of fungi collectively called the dimorphic fungi. In these fungi, conidia and hyphae are produced in their natural environments and can infect mammalian hosts. The dimorphic switch from yeast to hyphal form occurs in vivo induced by high mammalian body temperatures. Recent advances with molecular biology have begun to elucidate the mechanisms and importance of fungal dimorphism in these fungi.

### Dimorphic fungal pathogens – the shape shifters

Like *C. albicans*, the ability to dramatically alter morphology is also present in a small group of phylogenetically related fungi called the dimorphic fungi. These fungi include: *Blastomyces dermatitidis*, *Coccidioides immitis*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, and



*Penicillium marneffei*. These fungi are responsible for over one million new infections in the United States annually.<sup>133</sup> The ecology and epidemiology of these organisms are unclear; however, they are often found in soil associated with animal excreta and decaying wood.<sup>134</sup> In the environment, they produce hyphae and conidia (likely the infectious propagule). Once inhaled by mammalian hosts, like *Cryptococcus*, these fungi persist in macrophages and establish latent infections. Importantly, this group of fungi can change morphology from filamentous hyphal growth at ambient temperatures to yeast cells inside a mammalian host at 37°C. This ability to switch morphology has been hypothesized to be required for pathogenicity, and increasing molecular evidence has provided data to support the essential features of this hypothesis.

Some of the first data to support a link between fungal dimorphism and fungal virulence came from investigations on *H. capsulatum*, the causative agent of histoplasmosis. Mycelium treated with a sulfhydryl inhibitor, *p*-chloromercuriphenylsulfonic acid, a compound that prevents transition from yeast to hyphal form of the fungus, resulted in lack of disease establishment in a murine model.<sup>135</sup> With advances in molecular biology techniques available to study these dimorphic pathogens, molecular determinants of fungal dimorphism, and consequently pathogenicity, have begun to be identified.<sup>136,137</sup> For example, a small calcium-binding protein, *CBP1*, specific to the yeast phase of *H. capsulatum*, has been shown to be essential for virulence.<sup>138</sup> Two potential roles have been hypothesized for *CBP1* in *Histoplasma* virulence. First, *CBP1* could be performing a role in acquiring calcium from the extracellular environment, much like siderophores for iron acquisition. Second, *CBP1* could be a critical component of the various signal transduction cascades that rely upon calcium such as the calcineurin signaling pathway.

Importantly, it is clear that fungal dimorphism in these fungi is regulated by temperature.<sup>139-143</sup> At ambient temperatures dimorphic fungi exist as hyphal moulds similar to the previously discussed *Aspergillus* species. Indeed, the infectious propagule is the spore produced from the filamentous form of these moulds. Once inside a mammalian host, the change in temperature to 37°C stimulates a complex biochemical and molecular response in the fungus that leads to a drastic change in morphology from filamentous mould to globular yeast. In addition, in *Paracoccidioides brasiliensis* the conversion from conidia to yeasts is blocked by estrogen.<sup>144-146</sup> This finding appears to be clinically relevant as it may explain the increased incidence of this mycosis in males versus females. Thus, the question arises: Why have these fungi evolved the ability to change morphology from filamentous moulds at ambient temperatures to yeast at mammalian body temperatures? One hypothesis is that this transition is required in their ecologic niches, but to date no convincing evidence exists to confirm or refute this hypothesis.

Recently, a gene involved in regulating the morphologic switch between hyphal and yeast growth has been identified in *Blastomyces dermatitidis*.<sup>133</sup> This regulator, *DRK1* (dimorphism-regulating histidine kinase), is involved in a two-component signaling system that regulates dimorphism, virulence gene expression and virulence. A strain of *B. dermatitidis* lacking *DRK1* was unable to transition from hyphal morphology to yeast morphology at the permissive temperature (37°C), had altered cell wall composition, and a significant decrease

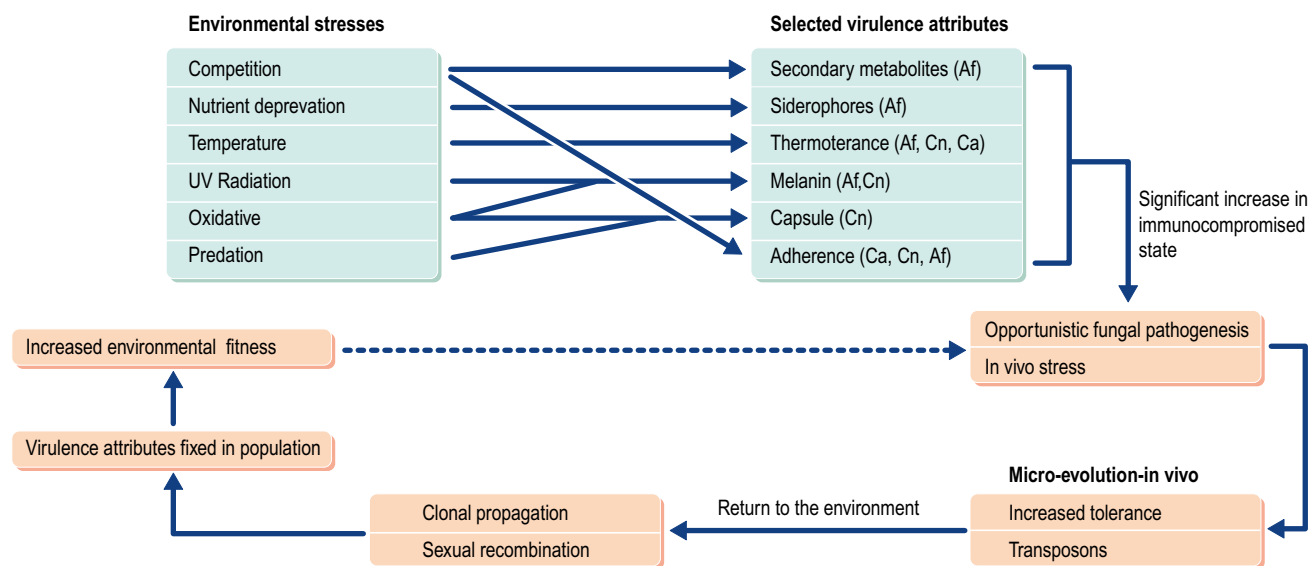
in sporulation. Importantly, *drk1* mutants were unable to cause disease in a mouse model of pulmonary infection.<sup>133</sup> To show the conservation of this important gene, *DRK1* was also silenced in *Histoplasma capsulatum*, and this *drk1* mutant strain was also unable to cause disease in a mouse model of histoplasmosis. These results strongly support the hypothesis that fungal dimorphism is required for fungal pathogenesis of mammals. Since humans lack histidine kinases like *DRK1*, fungal histidine kinases may serve as attractive targets for antifungal drug development. In addition, Nemecek and colleagues also hypothesize that *drk1* mutants could be used in the development of a vaccine against dimorphic fungal pathogens.<sup>133</sup>

## Fungal pathogenesis: where are we now and what does the future hold?

One recurring theme presented in this chapter is the lack of a “magic bullet” fungal virulence factor associated with human fungal pathogenesis. In contrast to this utopian notion, fungal pathogenesis of humans is primarily a multifactorial phenomenon that involves complex interactions between the invading fungal pathogen and recipient host to produce disease.<sup>147</sup> This is exemplified by the examples of known virulence attributes of the major human fungal pathogens presented in this review. For example, the ability to form a dense polysaccharide capsule is critical to *C. neoformans* pathogenesis but not *A. fumigatus* or *C. albicans*, and the ability to form invasive hyphae is critical for *A. fumigatus* but seemingly not for *C. neoformans* or *C. albicans*.

However, it is also evident that certain attributes are required for fungi to invade mammalian hosts, including: growth at mammalian body temperatures, resistance to oxidative stress, and ability to scavenge nutrients from a mammalian host. Importantly, the immune system status of the host is ultimately the determining factor that allows these fungi to cause disease. This fact alone strongly suggests that the majority of human fungal pathogens have not evolved specific virulence factors in the classic definition of the term. Instead, certain fungi have accumulated virulence attributes that likely arose from selection pressures in the fungi’s ecologic niches. These virulence attributes concomitantly allow these fungi to succeed in their natural ecologic environments and cause disease in immunocompromised hosts. In Figure 2-4 we present a possible model for the evolution of human fungal pathogenesis. In this model, we leave open the possibility that increased contact between fungi and immunocompromised humans may over time increase the ability of these fungi to cause disease in immunocompetent patients. While the *Cryptococcus* outbreak on Vancouver Island may suggest that this is plausible, one may argue that the lack of *C. albicans* virulence, given its intimate association with humans, disproves this possibility. Still, the unique reproductive and genetic mechanisms utilized by these fungi to create genetic diversity may be an important factor for consideration.

Further, while we have not focused on the host in this chapter, it is clear that the key risk factors that likely determine whether the majority of human fungal infections occur depend on the host immune system.<sup>148-151</sup> There are two major factors of invasive mycosis that cannot be ignored. First, except for commensalisms of some yeasts and the adaptation of certain



**Figure 2-4** Model of the evolution of human fungal pathogen virulence attributes. The model depicts possible stress encountered by fungi in their natural ecologic environments. These stresses are selection pressures that led to the development of attributes to allow the fungi to overcome these stresses. Consequently, with the arrival of immunocompromised patients, these attributes also allowed certain fungi the ability to persist in humans. Interactions with humans may lead to microevolution processes in vivo that have the potential to become fixed in the fungal population through sexual reproduction or clonal propagation. These traits may or may not confer an adaptive advantage on the fungus for future host colonization. Af, *Aspergillus fumigatus*; Cn, *Cryptococcus neoformans*; Ca, *Candida albicans*.

anthropophilic dermatophytes, fungi do not use humans as part of their life cycle. In fact, a fungal infection is an accidental encounter, which rarely leads to fungal transmission and therefore is most often an evolutionary dead end. However, fungi do participate in degrading the complex biomass and possess the tools to live off organic material. Thus, recent advances in medical technologies have created hosts whose impressive immune system has been abrogated in some way to allow establishment of fungal infection and production of disease. Second, we must clearly articulate that as clinicians we are primarily dealing with the ability of the fungi to create change in the normal homeostatic processes of the host or, in other words, disease.<sup>147</sup>

Fungal disease is best described in our view of the *Goldilocks paradigm* of host immunity. It occurs when there are host immune imbalances, either too little (invasive mycoses) to too much (immune reconstitution syndrome; IRS). These immune system perturbations can come from several sources. First, prematurity allows a variety of host issues to be circumvented. Second, there are both congenital and acquired immunodeficiencies which allow fungi to evade. Congenital defects in granulocyte function such as myeloperoxidase deficiencies and chronic granulomatous disease allow *Candida* and *Aspergillus*, respectively, to establish disease. Acquired defects can occur through iatrogenic or natural causes. For instance, cancer chemotherapy has produced profound neutropenias and risks for invasive fungal infections. With the nadir in blood counts and concomitant mucositis, it is clear that total absolute neutrophil counts under 500/mm<sup>3</sup> place the patient at risk for fungal infection and, without recovery of neutropenia, the inability to consistently cure infection with antifungals alone.

It is also clear that even with normal numbers of neutrophils, we can inhibit the immune system response with high doses of corticosteroid such as during graft-versus-host disease

or connective tissue diseases. Furthermore, as we continue to enlarge our indications for monoclonal antibody use, such as infliximab and alemtuzumab, cases of invasive mycoses will be uncovered. Along with these iatrogenically acquired immunosuppressive events, the world remains in the throes of the HIV epidemic. Despite highly successful antiretroviral therapy, viral destruction of immunity with lowering of CD4 cell counts to 200/mm<sup>3</sup> and the appearance of oral candidiasis to destruction of CD4 counts below 100/mm<sup>3</sup> is associated with the appearance of *Pneumocystis*, cryptococcosis or other endemic mycoses. Thus, it is absolutely clear that an intact cell-mediated immune system is critical to protection from invasive mycoses. Finally, with a further understanding of the human genome, we will likely uncover polymorphisms in specific immune system genes associated with a higher risk for fungal infections. These studies may help explain the “normal host” with an invasive fungal infection, such as those on Vancouver Island, or further stratify risk groups exposed to common fungi into higher or lower risk groups.

As important as the host immune response is for either protection from fungal invasion or elimination of an invading fungus, it has become clear that rapidly changing the immune response in the host can produce “too much” immunity.<sup>152</sup> This syndrome is called the immune reconstitution syndrome (IRS) and it can occur with established fungal infections and their management. For instance, during administration of HAART in a patient with HIV and mycoses or solid organ transplant recipient with mycoses and changing antirejection drug, as the fungus is being eliminated the exuberant immune response might damage the host with too many inflammatory cells at the site of infection.<sup>153,154</sup>

It is clear that despite great strides in the antifungal drug discovery arena (i.e., lipid products of amphotericin B, broad-spectrum azoles, and echinocandins), a further understanding

of fungal pathogens and their pathogenic mechanisms is needed to develop novel treatments and/or preventive strategies to better prepare for the inevitable development of antifungal resistance to current antifungal therapies. It is important to recognize that as we continue to gain understanding of the intricate details of what makes a human fungal pathogen, all of its basic pathogenic attributes are tightly linked to a powerful, changing host immune response, which range from simple structural barriers to innate factors and fluids to professional phagocytes to the highly complex and finely adjusted adaptive immune system. As our ability to successfully treat patients with what were formerly life-ending diseases continues to increase, the incidence of human fungal infections, and the morbidity and mortality associated with them, will continue to dramatically rise. As we gain a better appreciation of how these organisms colonize and cause disease in immunocompromised patients, we will increase our ability to thwart these life-threatening fungal infections.

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# Immunology

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## Introduction

The medically important fungi are a very diverse group, some of which are exceedingly prevalent in the environment. They provide a formidable challenge to the immune system. Even within species, variations in morphology with associated changes in surface antigens are common and not confined to the dimorphic fungi. For example, in susceptible hosts, inhaled *Aspergillus* conidia shed waxy surface coats, swell, and exhibit new surface antigens, then germinate to form hyphal filaments with further distinct antigens. These surface changes evoke distinct host humoral and cellular responses. Within tissues, *Candida albicans* may grow as yeastlike blastoconidia, pseudohyphae, and hyphae that differ in surface antigen expression. Moreover, *C. albicans* and other fungi can undergo switching of surface antigenic phenotypes in vivo.

A broad array of host defense mechanisms have evolved to protect humans against fungal invasion. Even lower invertebrates have the capacity to sense preserved molecular patterns on fungi (e.g.,  $\beta$ -glucans) and mount a protective response. Interestingly, features of many of these primitive innate defenses are conserved in humans. Vertebrates are also endowed with the capacity to mount adaptive immune responses, including antibody production and T cell-mediated immunity. Adaptive immunity is slower to develop but considerably more specific. The result of these efficient innate and adaptive defenses is that even the most pathogenic of the fungi usually induce only asymptomatic or self-limited infections in persons with an intact immune system. This is especially the case for the saprophytic, opportunistic fungi. Thus, the outcome of the host–fungus interaction usually depends on the status of host defenses and most serious fungal infections occur in persons with defects in one or more of these defenses. Awareness of the type of compromise afflicting a host enables the clinician to predict which mycoses are likely to occur (Table 3-1). Conversely, a patient without known immunocompromise who has a disseminated fungal infection usually warrants investigation for an underlying immunodeficiency, particularly acquired immunodeficiency syndrome (AIDS). Immunodeficiencies can be either acquired (e.g., due to medications such as corticosteroids) or congenital. Within the later category, it is becoming apparent that single nucleotide polymorphisms in

key immune response genes can increase susceptibility to certain infections.

For all mycoses, the clinical manifestations of disease can result from damage caused by the fungus as well as the host inflammatory response.<sup>1</sup> An extreme example of damage caused by the host response is mediastinal fibrosis due to *Histoplasma capsulatum*. Here, small numbers of fungi trigger an inflammatory response resulting in progressive life-threatening fibrosis of the mediastinum. At the other extreme, some patients with AIDS present with overwhelming cryptococcosis yet virtually no inflammatory response. However, immune reconstitution following initiation of antiretroviral therapy can then result in a life-threatening immune response inflammatory syndrome. Even in the absence of invasion, fungi can trigger immune-mediated hypersensitivity reactions that can present, for example, as asthma, extrinsic allergic alveolitis, or allergic bronchopulmonary aspergillosis.

For convenience, we have broken down the immune response into component parts (e.g., complement, antibody, neutrophils). However, the in vivo immune response is the result of an exceedingly complex integration of these parts, and divisions are somewhat arbitrary. T cell-mediated immunity seems to be especially important for host defense against *Cryptococcus neoformans*, *Pneumocystis jiroveci*, and the endemic dimorphic fungi, *Coccidioides immitis*, *H. capsulatum*, *Blastomyces dermatitidis* and *Paracoccidioides brasiliensis*. Protection against invasive candidiasis, aspergillosis, and zygomycosis is more dependent on intact neutrophil function.

## Non-immune factors in host defense against fungi

Although not usually considered a part of the immune system, a number of non-specific host factors form an important first line of defense against fungal invasion. These include the mechanical barrier provided by the skin and mucous membranes, competition for nutrients from the normal indigenous bacterial flora, and the mucociliary clearance system of the respiratory tract. The importance of these factors is illustrated by the association of disseminated candidiasis with the disruption of mechanical barriers by burns, surgical wounds or intravenous



**Table 3-1** Etiologic agents of systemic fungal infections associated with specific predisposing factors

Predisposing factor	Etiologic agent(s)
Traumatized skin and mucosal surfaces	<i>Candida</i> species
Neutropenia	<i>Candida</i> species (disseminated disease) <i>Aspergillus</i> species Agents of zygomycosis <i>Fusarium</i> species <i>Trichosporon</i> species <i>Pseudallescheria boydii</i> <i>Scedosporium</i> species
Impaired T cell-mediated immunity	<i>Candida</i> species (mucocutaneous disease) <i>Cryptococcus neoformans</i> <i>Histoplasma capsulatum</i> <i>Coccidioides immitis</i> <i>Pneumocystis jiroveci</i> <i>Paracoccidioides brasiliensis</i> <i>Penicillium marneffe</i>
Chronic granulomatous disease	<i>Aspergillus</i> species <i>Candida albicans</i> (disseminated disease)
Ketoacidosis	Agents of zygomycosis
Deferoxamine therapy	Agents of zygomycosis
TNF- $\alpha$ inhibitors	<i>Histoplasma capsulatum</i> <i>Aspergillus</i> species
Graft-versus-host disease	<i>Aspergillus</i> species

catheters, and with the inhibition of normal bacterial flora by broad-spectrum antibiotics.<sup>2</sup> In serum, chelation of iron and other essential heavy metals restricts the growth of many fungi. The association of zygomycosis (mucormycosis) with therapy with the iron chelator deferoxamine is thought to be due to the ability of the causative fungi to use iron-saturated deferoxamine as a siderophore.<sup>3-5</sup> Similarly, ketoacidosis may predispose patients to zygomycosis by making iron more readily available to the fungus. In addition, incompletely characterized serum and cerebrospinal fluid (CSF) factors have been reported to inhibit fungal growth.<sup>6,7</sup>

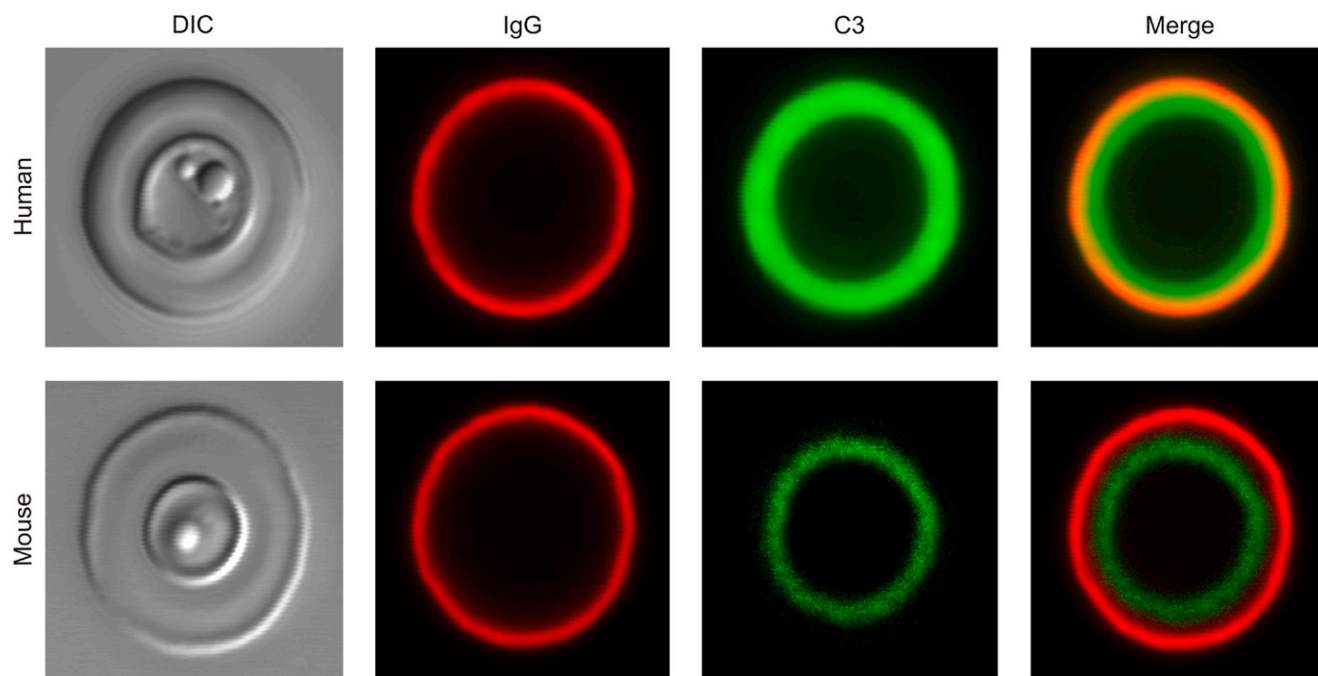
## Complement

The complement system is made up of more than 30 proteins found in blood and extracellular fluid.<sup>8</sup> These complement components can be activated in a cascade-like fashion by way of three pathways, designated the classic, alternative, and lectin pathways, each of which results in activation of C3.

Components of each activation pathway are proenzymes. The cleavage of each proenzyme generates a serine protease that cleaves the next proenzyme in the sequence. Initiation of the classic pathway occurs by binding of C1q to the Fc portion of IgG or IgM antibody bound to antigen. Initiation of the alternative pathway does not require antibody. Instead, spontaneous hydrolysis of some circulating C3 leads to generation of a fluid-phase C3 convertase, C3(H2O)Bb, capable of cleaving C3. By this mechanism, small amounts of C3b are probably continuously generated. Further activation and amplification only occur if some C3b so generated is bound to an activating particulate surface. Such surfaces favor the binding of factor B and the formation of solid-phase C3 convertase.<sup>9</sup> On non-activating surfaces, binding of factor H promotes cleavage of C3b by factor I to yield enzymatically inactive iC3b. Activation of the lectin complement pathway occurs when pathogen recognition by mannose-binding lectin (MBL) or ficolins trigger MBL-associated serine proteases (MASPs).<sup>8</sup> MBL appears particularly relevant for host defenses against fungi as exposed mannose residues are frequently present on fungal surfaces.<sup>10,11</sup>

Activation of the terminal complement components (C5–C9) by either the classic or alternative pathways can lead to the assembly of a pore-forming membrane attack complex on target membranes and the lysis of some bacteria and viruses.<sup>11</sup> Such direct killing of pathogenic fungi has not been demonstrated, presumably because of the thick fungal cell wall. However, activation of the complement system has a number of other functions, some of which are implicated in host defense against fungi. C3 fragments bound to fungal surfaces act as opsonins that promote binding and phagocytosis of the fungi by leukocytes bearing the appropriate complement receptors. In addition, cleavage of C3, C4, and C5 releases soluble proinflammatory fragments, C3a, C4a, and C5a. These anaphylotoxins cause release of histamine and other mediators from mast cells and basophils. C5a, the most potent, is also chemotactic for neutrophils and enhances neutrophil migration across the endothelium. Last, complement activation by means of the classic pathway can also promote the clearance of potentially harmful immune complexes.

Fungi are generally potent activators of the alternative complement pathway.<sup>12</sup> Studies of patients with cryptococcosis and of mice infected with *C. neoformans* suggest that complement activation occurs in vivo. Alternative pathway components were found to be depleted in patients with cryptococemia.<sup>13</sup> C3 has been detected on *C. neoformans* isolated from the skin, but not the CSF of patients with cryptococcal meningitis.<sup>14,15</sup> Similarly, in a murine model Truelsen et al<sup>16</sup> found readily detectable C3 on cryptococci from liver and lung but not on cryptococci from brain tissue. Absence of complement-mediated opsonization in the central nervous system is one possible factor explaining the predilection of *C. neoformans* to cause infection at this site. Interestingly, there are species-related differences regarding C3 deposition (Fig. 3-1). Activation by human serum results in C3 bound at or very near the capsular edge whereas with mouse serum, C3 is buried beneath the capsular surface, and thus not available to interact with complement receptors.<sup>17</sup> Complement likely has two main functions in host defense against cryptococcosis: opsonization and induction of inflammation. In vitro binding and phagocytosis of serum-opsonized encapsulated



**Figure 3-1** Species-specific differences in binding of C3 to encapsulated *C. neoformans*. Differential interference contrast and confocal microscopy images of *C. neoformans* following incubation in human and mouse serum. Red color demonstrates the capsule rim whereas the green color demonstrates C3. Note human C3 is located at the capsular surface whereas mouse C3 is subcapsular (reproduced with permission from Gates and Kozel.<sup>17</sup>).

cryptococci by macrophages is complement dependent and can be blocked by antibodies to the CR3, CR4, and CR1 complement receptors.<sup>18</sup>

All three complement pathways can be activated by *C. albicans*.<sup>11,12,19</sup> Normal human serum contains IgG anti-mannan antibodies which can activate the classic pathway. MBL has been shown to bind to *C. albicans* both in vitro and in vivo.<sup>11</sup> Unlike the case with *C. neoformans*, most C3 bound to *C. albicans* is in the form of C3b.<sup>12</sup> Also in contrast to encapsulated cryptococci, binding of *Candida* blastoconidia to macrophages in the presence of normal serum likely involves Fc, as well as complement receptors. Consistent with the function of C5a as a neutrophil chemoattractant and the known importance of neutrophils in defense against disseminated candidiasis, C5-deficient mice are also more susceptible to disseminated disease.<sup>20</sup> Interestingly, as well as providing a surface for complement activation, *C. albicans* has been shown to have CR2 and CR3 complement receptor-like activities, as manifest by their capacity to bind C3d- and iC3b-coated sheep erythrocytes.<sup>21</sup>

Other pathogenic fungi have also been shown to activate complement.<sup>12</sup> *Aspergillus* conidia and hyphae exposed to normal serum bind C3 fragments (C3b and iC3b). Complement activation by *Aspergillus* antigens has been postulated to contribute to allergic asthma by generation of the complement anaphylatoxins C3a and C5a.<sup>22</sup> Activation of complement has also been demonstrated in vitro for a number of other pathogenic fungi including *H. capsulatum*, *C. immitis*, *B. dermatitidis*, *P. brasiliensis*, *Sporothrix schenckii* and *Trichophyton* species, but the role of complement in host defense against these fungi is less clear. As discussed later, unopsonized *H. capsulatum* and *B. dermatitidis* bind to phagocytes by way of complement receptors.<sup>23-25</sup>

In addition to the components of the complement system, other soluble molecules participate in innate antifungal host defenses. The long pentraxin, PTX3, is a secreted pattern recognition molecule which binds to *Aspergillus* conidia.<sup>26</sup> PTX3-deficient mice have impaired phagocyte recognition of *Aspergillus fumigatus* and are susceptible to invasive pulmonary aspergillosis.<sup>26</sup> Saliva contains anticandidal peptides, including histatins and  $\beta$ -defensins,<sup>27</sup> which may help explain the predisposition of patients with xerostomia to oral candidiasis.

## Antibody

Recent studies have emphasized the complexities of the interaction of humoral and cell-mediated immunity, and the somewhat artificial nature of this simple division of the adaptive immune response.<sup>28</sup> Nevertheless, for many of the medically important fungi, an important role for specific antibodies in natural immunity remains unproven. In contrast to patients with impaired cell-mediated immunity or neutropenia, those with hypogammaglobulinemia are not particularly predisposed to the development of invasive fungal infections and studies correlating the presence of specific antibody with protection have yielded conflicting results. In addition, in animal models of aspergillosis, blastomycosis, and coccidioidomycosis, studies to date have not shown any beneficial effect from administration of immune serum.<sup>29</sup> Moreover, B cell-deficient animals do not appear to be more susceptible to histoplasmosis<sup>30</sup> and mucosal candidiasis.<sup>31</sup> Nevertheless, for *C. albicans*, *C. neoformans* and *H. capsulatum*,<sup>32</sup> in vitro studies demonstrating enhanced effector cell activity in the presence of antibody and animal model studies showing modification of disease with antibody administration suggest that, whatever the importance



or complexities of the role of antibody in natural infection, passive administration of particular specific antibodies could be beneficial to patients with these mycoses. Of note, work with monoclonal antibodies against both *C. albicans* and *C. neoformans* has shown that, depending on fine specificity and isotype, antibodies may be protective, neutral or actually disease enhancing, a finding that may help explain the inconsistent results of earlier studies with polyclonal sera.

Experimental immune sera to *C. albicans*, depending on the preparation used and route of inoculation, have been shown to contain antibodies to more than 50 different antigenic components.<sup>33</sup> Three types of studies suggest humoral immunity plays some role in protection against disseminated candidiasis. First, mice depleted of IgM-bearing B cells have been shown to have enhanced susceptibility to systemic candidiasis.<sup>34</sup> Second, for some but not all *Candida*-specific antibodies, a correlation between the presence of antibody and protection has been found.<sup>35,36</sup> Third, some studies have found administration of specific antibody, including those against the heat shock protein (HSP) 90 and mannan epitopes, to be beneficial in animal models of disseminated infection.<sup>37,38</sup>

Mechanisms of protection from mucosal candidiasis may be site specific. While cell-mediated immunity is clearly important for protection against oropharyngeal and esophageal candidiasis,<sup>39</sup> and vaginal disease may result from an overexuberant innate response,<sup>40</sup> there is only limited evidence for an important role for humoral immunity at either mucosal site. IgA deficiency is not associated with more severe mucosal disease. There were no differences in *Candida*-specific antibody levels in saliva of HIV-seropositive individuals with and without oral candidiasis.<sup>41</sup> In addition, levels of vaginal *Candida*-specific IgA and IgG are similar in women with and without candidiasis, and the presence of antibody does not protect from recurrent infection.<sup>42</sup> Nevertheless, some studies have demonstrated protection against *Candida* vaginitis in rats by passive administration of antibody specific for aspartyl proteinase and mannan antigens.<sup>43,44</sup>

Most patients with cryptococcosis have defects in cell-mediated immunity, but some evidence suggests humoral immunity may also play some role in protection. There are reports of cryptococcosis in patients with hyper-IgM syndrome and hypogammaglobulinemia.<sup>45-47</sup> In patients with cryptococcal meningitis, specific antibody is a favorable prognostic sign, and the appearance of antibody in the CSF may accompany recovery.<sup>48,49</sup> Nevertheless, some studies suggest the natural antibody response to *C. neoformans* is often dominated by anticapsular antibodies that are not opsonic and may not be protective.<sup>50,51</sup>

Although earlier studies with polyclonal sera were inconclusive, several groups of investigators have now shown beneficial effects of administration of certain monoclonal antibodies against the glucuronoxylomannan component of the cryptococcal polysaccharide capsule, as well as a peptide mimetic of such an antibody.<sup>52-55</sup> Both isotype and specificity are important determinants of efficacy and, as with *C. albicans*, both protective and non-protective antibodies have been described. Class switching of a non-protective IgG3 antibody to an otherwise identical IgG1 antibody caused it to become protective.<sup>56</sup> The importance of fine specificity is illustrated by the fact that of two IgM antibodies derived by somatic mutation from the same B cell, one was protective and the other was not.<sup>57</sup> More

recently, monoclonal antibodies to a cell surface histone-like protein of *H. capsulatum* have been shown to prolong survival of mice when given prior to intranasal infection.<sup>32</sup> Prolonged survival was associated with increased IL-4, IL-6, and IFN- $\gamma$  and reduced inflammation in the lungs, and reduced fungal burden. In vitro, the antibody increased phagocytosis and inhibition of growth of *H. capsulatum* by murine macrophages.

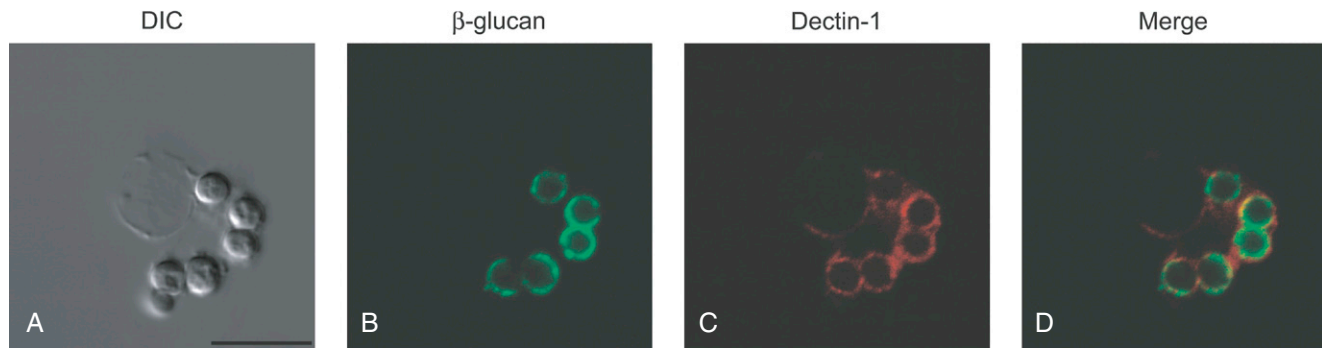
The mechanisms whereby particular antibodies modify the course of fungal infections are under investigation. Antibodies to *C. albicans* mannan may interfere with adhesion, and have been shown to lead to rapid complement deposition on fungal cells, thereby enhancing the candidicidal activity of phagocytes.<sup>58,59</sup> Antibodies against HSP90 bind fungal HSP on the *Candida* cell surface and have direct antifungal activity, although it is also possible that some benefit may derive from inhibition of human HSP, that is involved in pathways leading to circulatory shock.<sup>38</sup>

Anticryptococcal antibodies can be potent opsonins<sup>60,61</sup> and have been shown to enhance cryptococcal antigen presentation and the activity of neutrophils, mononuclear cells, and natural killer cells against *C. neoformans*.<sup>62-65</sup> Anticapsular antibodies also cause clearance of potentially harmful glucuronoxylomannan (GXM), reduce release of GXM from the capsule,<sup>66</sup> and abrogate many of the immunosuppressive effects of GXM (reviewed in reference 67). However, a body of data now suggests a complex interaction between protective antibodies to GXM and cellular immunity. The beneficial effects of an IgG1 antibody in immunocompetent mice were not seen in severe combined immunodeficiency (SCID) (T cell- and B cell-deficient) mice, CD4 T cell-deficient mice, or IFN- $\gamma$  knockout mice.<sup>68</sup> Subsequently antibody-mediated protection was found to be associated with largely downregulatory changes in lung cytokines and to involve, as well as both Th1 and Th2 cytokines, B cells, iNOS, and mouse genetic background.<sup>69-74</sup> These and other observations have led Casadevall and Pirofski to propose that antibody may have proinflammatory effects early in infection but also beneficial anti-inflammatory effects that reduce immune-mediated pathology in established infection.<sup>28</sup>

Specific antibodies may also play a critical role in the pathogenesis of allergic responses to inhaled fungi. For example, IgE-mediated reactions to fungal allergens may play a part in some asthmatic attacks;<sup>75</sup> fungal-specific IgE and IgG may be involved in the pathogenesis of chronic rhinosinusitis in some patients;<sup>76</sup> and precipitating antibodies to fungal antigens are responsible for some of the manifestations of extrinsic allergic alveolitis through the formation of immune complexes. This topic is also discussed in Chapter 25.

## Non-opsonic recognition of fungi by phagocytes

As noted above, opsonization, particularly with complement and antibody, plays a critical role in facilitating host recognition of invading fungi. However, even in the absence of opsonization, phagocytes will recognize surface-exposed ligands on most fungal pathogens. (Due to its antiphagocytic capsule, *C. neoformans* is the notable exception.) Following host cell recognition, two types of responses, which are not mutually exclusive, can occur. The first response leads to actin-dependent



**Figure 3-2** Dectin-1 is recruited to alveolar macrophage phagosomes containing *A. fumigatus* conidia. Differential interference contrast and confocal microscopy images of mouse alveolar macrophages following in vivo challenge with *A. fumigatus* swollen conidia. Cells were incubated with green-labeled antibody against  $\beta$ -glucan and red-labeled antibody against dectin-1 (Reproduced with permission from Hohl TM, VAN Epps HL, Rivera A, et al. *Aspergillus fumigatus* triggers inflammatory responses by stage-specific  $\beta$ -glucan display. *PLoS Pathogens* 1: e30, 2005).

phagocytosis (internalization) of the fungal cell. The second response leads to stimulation of phagocyte signaling pathways. Importantly, the antifungal response appears to be critically dependent upon which receptors the pathogen stimulates.

Fungal cell walls are rich in  $\beta$ -glucans and mannans. Dectin-1 is expressed on nearly all phagocyte populations and mediates uptake of fungi with exposed  $\beta$ -glucans<sup>77-80</sup> (Fig. 3-2). However, for some fungi, the  $\beta$ -glucans are mostly in the inner cell wall, masked by mannans,  $\alpha$ -glucans or capsule, and may not be available to interact with dectin-1.<sup>81,82</sup> The cytoplasmic tail of dectin-1 contains an immunoreceptor tyrosine activation motif which is necessary for its signaling properties.<sup>77,78</sup> The specific ligand for dectin-2 is less well defined, although binding of dectin-2 to *C. albicans* hyphae has been demonstrated.<sup>83</sup> Mannans or mannoproteins are surface exposed on most fungal cell walls, and for some fungi are secreted.<sup>10,84</sup> Mannosylation of proteins can occur via either N-linkages or O-linkages. Exposed mannose groups are recognized by mannose receptors present on a wide variety of cell types, particularly dendritic cells (DC) and macrophages.<sup>10,85,86</sup> As with dectin-1, mannose receptors are competent to mediate both phagocytosis and signaling responses. The major mannose receptors are the macrophage mannose receptor and DC-SIGN.

The original association of Toll with antimicrobial host defenses in *Drosophila* was made when it was noted that flies deficient in Toll were abnormally susceptible to challenge with *A. fumigatus*.<sup>87</sup> Soon thereafter, based on their sequence similarities in the cytoplasmic portions, the Toll-like receptors (TLR) were discovered.<sup>88,89</sup> TLR represent a family of conserved proteins that mediate intracellular signaling responses to conserved pathogen-associated molecular patterns. The major function of TLR appears to be signaling rather than phagocytosis, although TLR can act cooperatively with phagocytic receptors including mannose receptors and dectin-1.<sup>77,79</sup> Ultimately, the cytokine and chemokine response of the phagocyte to fungal stimulation depends in large measure on which of the many innate immune receptors are stimulated. In vitro, TLR2 appear to be the most important of the TLR for signaling responses to fungi, although roles for TLR4 and TLR9 have also been described.<sup>88-91</sup> However, in vivo, perhaps due to redundancy in the immune system, mice deficient in individual TLR generally have had no or only relatively minor increases in susceptibility to fungal challenge.<sup>88-92</sup>

Some fungi appear to be able to exploit phagocytic receptors to gain entry into the cell. This may facilitate intracellular parasitism, as it allows the pathogen to avoid many of the effector pathways that would otherwise be triggered in response to opsonins, mannans or  $\beta$ -glucans. Entry of *H. capsulatum* to macrophages and neutrophils is mediated by an interaction between HSP60 on the fungal surface with CD18 present on the phagocytic surface.<sup>23,24,93</sup> In contrast, even though DC express CD18, they instead utilize very late antigen-5 (VLA-5) to phagocytose *H. capsulatum*.<sup>94</sup> *B. dermatitidis* uses a cell wall protein, BAD1, to gain access to macrophages via CR3 and trigger an antiinflammatory program which fosters pathogen survival.<sup>25,95</sup> Table 3-2 summarizes many of the known ligands on major fungal pathogens that are recognized by specific phagocytic receptors.

## Neutrophils and eosinophils

Neutrophils, also known as polymorphonuclear granulocytes, constitute primary effector cells in acute inflammation. Present in large numbers in circulating blood, they are rapidly recruited to sites of infection by a carefully regulated series of events that features adhesion to the vascular endothelium followed by migration across the endothelium and through tissue.<sup>96</sup> Microbial products, complement components (especially C5a), chemokines (especially IL-8), and arachidonic acid metabolites act on endothelial cells and neutrophils to initiate this series of events. Once at the inflammatory site, binding to microbes occurs by means of specific neutrophil receptors and is facilitated if the microbe is opsonized by C3 or IgG. However, binding may also occur in the absence of opsonins because of the presence of ligands on the microbial surface (e.g., mannose and  $\beta$ -glucan residues) that are recognized by neutrophil receptors. After binding, actual phagocytosis (internalization) of the organism usually occurs.

Killing of microorganisms can occur by oxidative or non-oxidative mechanisms.<sup>97</sup> Oxidative mechanisms refer to processes dependent on the respiratory burst, whereby molecular oxygen is reduced to superoxide anion. Most of the superoxide is dismutated to hydrogen peroxide.  $H_2O_2$  has relatively weak antimicrobial activity. However, in a reaction catalyzed by neutrophil granule enzyme myeloperoxidase,  $H_2O_2$  can react

**Table 3-2** Examples of receptors and ligands involved in the binding of unopsonized fungi to phagocytes

Pathogen	Fungal Ligand(s)	Phagocytic Receptor(s)
<i>Aspergillus fumigatus</i>	Mannans, $\beta$ -glucans	DC-SIGN, dectin-1
<i>Blastomyces dermatitidis</i>	BAD1	CR3, CD14
<i>Candida albicans</i>	Mannans, $\beta$ -glucans	Mannose receptors, dectin-1
<i>Coccidioides posadasii</i>	Mannans, $\beta$ -glucans	Mannose receptors, dectin-1
<i>Cryptococcus neoformans</i>	Glucuronoxylomannan*	TLR2, TLR4, CD14, CD18, Fc $\gamma$ RII
<i>Histoplasma capsulatum</i>	HSP60	CD18, VLA-5
<i>Pneumocystis jiroveci</i>	Mannans, $\beta$ -glucans	Mannose receptors, dectin-1

\*Unopsonized *C. neoformans* generally is not recognized by phagocytes. Shed glucuronoxylomannan is recognized by the indicated receptors.

with a halide ion to form oxidants (e.g., hypochlorous acid) with potent microbicidal activity. Monocytes and macrophages will also undergo a respiratory burst on stimulation, although at a level of activity considerably less than that seen in neutrophils. Moreover, mature macrophages lack myeloperoxidase. Neutrophil granules contain substances that can mediate oxygen-independent microbicidal activity, including defensins, lactoferrin, and calprotectin. During neutrophil activation, degranulation occurs with release of granule contents into the phagolysosome and extracellular space.

Clinically, there is a strong association of neutropenia with disseminated candidiasis and invasive aspergillosis. Undoubtedly, this association is a reflection of not only the paramount importance of neutrophils in host defenses against these two mycoses but also the frequency with which exposure to these ubiquitous fungi occurs. Many other mycoses, including zygomycosis, fusariosis and pseudallescheriasis, although still relatively rare, nevertheless occur with greatly increased prevalence in neutropenic hosts. The association of neutropenia with mycoses has prompted research into the interactions of neutrophils with a variety of fungi, in particular *Candida* and *Aspergillus*.

In vitro, human neutrophils can kill *C. albicans* yeast cells, pseudohyphae and hyphae, and *A. fumigatus* hyphae.<sup>98-100</sup> In contrast, *A. fumigatus* conidia, which are the inhaled form of the organism, are resistant to neutrophil killing despite being readily phagocytosed.<sup>101</sup> *Candida* and *Aspergillus* hyphae are too large to be ingested by neutrophils; however, groups of neutrophils can attach to the fungal surface and kill it.<sup>99,100</sup> Hyphae of both fungi stimulate neutrophils to undergo a respiratory burst and degranulate. The oxidants generated

(e.g., hydrogen peroxide and hypochlorous acid) and granule products released (e.g., defensins) are fungicidal.<sup>101,102</sup>

The importance of neutrophils in defense against *C. albicans*, *A. fumigatus* and other catalase-positive fungi is highlighted by the frequency of these mycoses (20% in one large series) in patients with chronic granulomatous disease (CGD), an inherited disorder of the NADPH oxidase.<sup>103</sup> Neutrophils from CGD patients are defective in their ability to generate a respiratory burst and produce only scant amounts of microbicidal oxidants. Consequently, CGD neutrophils have difficulty killing catalase-positive organisms, including *Aspergillus* and *Candida* species. With catalase-negative organisms, CGD neutrophils are able to make up for their deficient H<sub>2</sub>O<sub>2</sub> production by use of the H<sub>2</sub>O<sub>2</sub> produced by the organisms. Catalase-positive organisms, by degrading the H<sub>2</sub>O<sub>2</sub> they produce, deprive the phagocyte of its endogenous H<sub>2</sub>O<sub>2</sub>.<sup>104</sup>

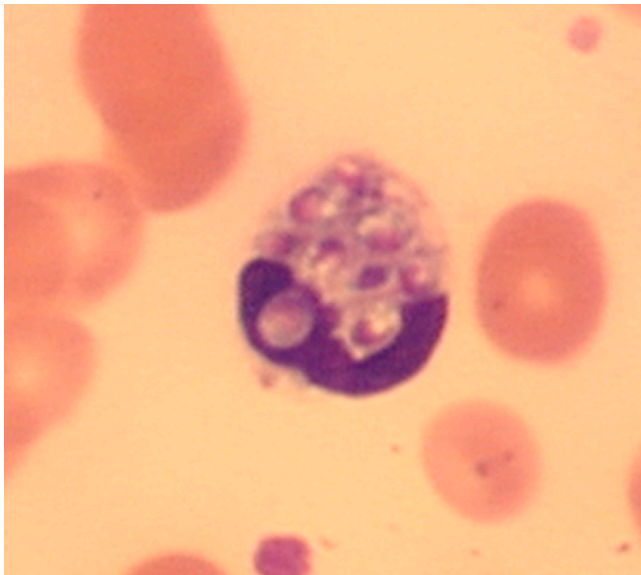
Neutrophils possess cytokine receptors, and stimulation of neutrophils with the appropriate cytokines can augment fungal killing,<sup>105,106</sup> suggesting feedback mechanisms whereby stimulated mononuclear cells produce cytokines, which in turn activate neutrophils for more effective fungal killing. In vivo administration of recombinant IFN- $\gamma$  to CGD patients has been shown to significantly reduce the incidence of serious infections. Neutrophils from IFN- $\gamma$ -treated CGD patients acquire the capacity to damage *A. fumigatus* hyphae.<sup>107</sup> Conversely, candidacidal activity of neutrophils was impaired by the antiinflammatory cytokines IL-4 and IL-10,<sup>108</sup> a process that may help limit the damage that can be caused by an overly exuberant inflammatory response. When stimulated with *C. albicans* and *A. fumigatus*, neutrophils secrete both proinflammatory and antiinflammatory cytokines.<sup>109-111</sup>

The pathology of disseminated candidiasis and aspergillosis features angioinvasion. This finding has stimulated study of the interaction of *C. albicans* and *A. fumigatus* with vascular endothelium and how neutrophils affect this interaction. Multiple adhesins have been described that facilitate this process, including integrin analogs (fungal proteins exhibiting antigenic and functional similarity to mammalian integrins) that recognize integrin ligands on endothelial cells.<sup>112,113</sup> Many of these same receptors likely mediate adherence of *C. albicans* to epithelial cells.

Compared with neutrophils, less is known about the contribution of eosinophils to host responses to invading fungi. Eosinophils are commonly seen in association with allergic fungal diseases, including sinusitis and asthma. In such cases, it has been postulated that eosinophils are deleterious due to their release of toxic products, including major basic protein and eosinophil peroxidase.<sup>114</sup> In vitro, extracts from the environmental fungi *Alternaria alternata* and *Penicillium notatum* induced exocytosis in eosinophils from normal individuals.<sup>114</sup> An eosinophilic response is occasionally found in humans with mycoses, particularly with coccidioidomycosis.<sup>115</sup>

## Mononuclear phagocytes

Mononuclear phagocytes are central to a protective immune response to a number of the most important fungal pathogens. Monocytes, resident macrophages, and DC form a part of the first line of innate cell-mediated immunity. In addition, mononuclear phagocytes, particularly DC, are critical in linking the



**Figure 3-3** *H. capsulatum* within a human peripheral blood monocyte. Peripheral blood smear from a patient with AIDS and progressive disseminated histoplasmosis. Numerous intracellular *H. capsulatum* yeast cells are apparent within a monocyte.

innate and adaptive immune responses by initiating and propagating specific T cell-mediated immune responses. Once activated, T cells can then secrete cytokines to activate phagocytes to become more potent effector cells. Many fungi, particularly *C. neoformans* and *H. capsulatum*, appear adapted for survival within phagocytes (Fig. 3-3).

## Dendritic cells

Dendritic cells (DC) are present in all organs of the body where they act as sentinels. Upon exposure to antigens and a maturation signal, DC migrate to lymphatic tissue where they mature, secrete cytokines and initiate cell-mediated immune responses.<sup>116-118</sup> The interaction of DC with naïve T cells in the lymph node not only results in generation and expansion of antigen-specific T cells but also polarizes the T cells to differentiate into Th1, Th2 or regulatory T cells. As such, DC are key bridges between innate and adaptive immunity. Not surprisingly then, a variety of studies have demonstrated the critical importance of DC in initiating CD4+ and CD8+ T cell responses against fungal pathogens.<sup>89,119</sup>

Although their primary function is thought to be initiation of adaptive immune responses through the process of antigen presentation, DC are also phagocytic effector cells. Fungal pathogens for which DC phagocytosis and/or antifungal activity have been documented include *C. albicans*, *H. capsulatum*, *A. fumigatus*, *C. neoformans* and *C. posadasii*.<sup>94,120-125</sup> Opsonization with complement or antibody is required for DC phagocytosis of encapsulated *C. neoformans*, whereas for the other fungi, ligands present on the fungal surface are recognized by DC receptors. The mechanisms by which DC kill fungal cells have not been well defined, although one study implicated lysosomal hydrolases.<sup>126</sup> DC can mature, secrete cytokines and present fungal antigens following phagocytosis. The morphotype of the fungus can influence the nature of

the immune response. DC phagocytosis of *C. albicans* yeast cells and *A. fumigatus* conidia resulted in a Th1-type response whereas the hyphal forms of these fungi stimulated DC to induce a Th2-type response.<sup>121,123</sup>

## Macrophages

Macrophages originate from circulating monocytes following recruitment to tissue and subsequent differentiation. The antifungal properties of macrophages are greatly influenced by the anatomic site in which differentiation occurs and also the state of activation of the macrophage. Activation of macrophages with lymphocyte-derived cytokines, particularly interferon- $\gamma$ , may impart fungicidal properties upon these phagocytes. This presumably accounts for the critical importance of cell-mediated immunity in the control of many fungal pathogens.

With the notable exception of *C. albicans*, for most fungi that cause systemic disease, initial exposure occurs by inhalation of airborne fungal cells. Therefore, bronchoalveolar macrophages constitute a particularly important component of host defense. Bronchoalveolar macrophages inhibit the growth of a number of fungi and have usually been found to have greater antifungal activity than other macrophage populations. In vitro, bronchoalveolar macrophages can kill the conidia but not the hyphae of *A. fumigatus*. Conversely, neutrophils cannot kill ungerminated conidia but can kill hyphae.<sup>127,128</sup> In vivo, these two lines of phagocyte defense may combine to prevent the establishment of infection. Conidia that escape killing by resident bronchoalveolar macrophages and germinate will be susceptible to attack by recruited neutrophils. Bronchoalveolar macrophages have been shown to have activity against other fungal species, including *C. neoformans*, *Rhizopus arrhizus*, and *B. dermatitidis*.<sup>128-130</sup> In contrast, following ingestion by alveolar macrophages, *H. capsulatum* microconidia, the inhaled form of the organism, undergo phase-transition into yeast cells and then replicate.<sup>131</sup>

As with neutrophils, monocytes and macrophages exert antifungal activity by both oxidative and non-oxidative mechanisms.<sup>132</sup> Reactive nitrogen intermediaries, including nitric oxide, produced by activated macrophages play a key role in mediating the antifungal activity of murine macrophages.<sup>133</sup> However, because the quantities of reactive nitrogen intermediaries made by activated human macrophages are considerably lower, it is uncertain whether this antifungal mechanism is operative in human cells.<sup>134</sup> The non-oxidative mediators of antifungal activity are less well defined, but likely include degradative enzymes and antimicrobial peptides. Enzymes, including chitinases, that may play a role in the degradation of the fungal cell wall have been described.<sup>135</sup> Acidification of the phagosome seems to occur to at least some degree following phagosome-lysosome fusion, but may be a “double-edged sword.” While a low pH promotes optimal activity of lysosomal hydrolases, it also makes iron available to the fungus.<sup>136,137</sup> *H. capsulatum* has developed mechanisms whereby it can maintain a phagosomal pH of about 6.5, thus allowing the organism to obtain iron and to minimize the activity of macrophage lysosomal hydrolases.<sup>138</sup> Interestingly, for both *H. capsulatum* and *C. neoformans*, agents such as chloroquine that raise phagolysosomal pH were found to significantly enhance the antifungal activity of human macrophages.<sup>139,140</sup> For *H. capsulatum* the effect of chloroquine was mediated by



restriction of iron availability at a higher pH. For *C. neoformans* the effect of chloroquine was independent of iron deprivation and related to the poor growth of *C. neoformans* at higher pH.

## T cell-mediated immunity

### The specific CD4 T cell-mediated immune response

Specific cell-mediated immunity is critical for a protective immune response to *C. neoformans* and the dimorphic fungi and is involved in protection against dermatophyte infections. Cell-mediated immunity, rather than intact neutrophil function, is also of primary importance for protection against oropharyngeal and esophageal candidiasis. Evidence for the importance of specific cell-mediated immunity comes from clinical observation and animal studies. These mycoses are seen with increased frequency in patients with AIDS, lymphomas, and sarcoidosis, and in those taking immunosuppressive medications such as corticosteroids, cyclophosphamide or azathioprine that depress T cell-mediated immunity. The high incidence of fungal infections in HIV-infected patients is particularly striking. HIV infects CD4<sup>+</sup> lymphocytes and mononuclear phagocytes, the two cell types whose interactions are central to the cell-mediated immune response.

Development of a specific CD4 T cell-mediated immune response requires antigen-presenting dendritic cells to process and present fungal antigen(s) to T lymphocytes. Exogenous antigens such as fungi are taken up into acidic vesicles of the endosome-lysosome pathway and processed into peptide fragments. These peptides bind to major histocompatibility complex (MHC) class II molecules within the vesicles and the MHC-peptide complexes are then expressed on the cell surface where they may be recognized by antigen-specific CD4 Th (T helper) cells. The T cell receptor binds to the peptide that lies within a groove in the MHC molecule and to polymorphic determinants on the MHC, whereas the CD4 co-receptor binds to a separate site on the MHC class II molecule. For T cell activation to occur, a second signal must be provided by co-stimulatory molecules on the antigen-presenting cell (APC), such as CD80 and CD86. The co-stimulatory molecules interact with corresponding receptors on T cells. Other factors, including cytokines secreted by the APC (see below), influence the nature and magnitude of the resultant T cell response. Activation of T cells leads to proliferation and clonal expansion through upregulation of the expression of IL-2, the principal T cell growth factor, and of the IL-2 receptor. Clonal expansion of antigen-specific T cells provides an enlarged pool of effector cells and, subsequently, of antigen-specific memory cells that can initiate a more rapid response on subsequent exposure to the same antigen. Activation also causes expression of other cytokines and of surface molecules involved in the effector function of these Th cells.

### Th1- and Th2-type responses

Different subsets of CD4 Th cells exist that secrete different patterns of cytokines. Naive T cells, depending on factors such as co-stimulatory signals from antigen presenting cells, cytokine

milieu, and antigen dose, may become polarized, secreting predominantly IL-2 and IFN- $\gamma$  (Th1 pattern) or predominantly IL-4, IL-5, and IL-13 (Th2 pattern). This differential development is controlled by specific sets of transcription factors.<sup>141</sup> Once established, patterns of response may be maintained by cross-regulation whereby, for example, IL-4 and IFN- $\gamma$  inhibit each other's production. Originally defined in cloned murine T cell lines, T cells that fit these patterns of cytokine release have been defined in many human and experimental animal immune responses, including those to fungi. Furthermore, earlier evidence for the importance of Th1-type responses in protection from fungal infection in murine models has been supported by characterization of immune responses in patients. Thus, although the Th1 and Th2 paradigm is undoubtedly an oversimplification, and is now complicated by the recognition of further subsets of regulatory T cells (see below), it has greatly advanced our understanding of protective and non-protective responses to fungal infection.

Different immune responses are mediated by Th1 and Th2 patterns of cytokine release: IFN- $\gamma$  and IL-2 activate macrophages and cytotoxic T and natural killer (NK) cells, respectively, for clearance of intracellular organisms. In contrast, Th2 cytokines favor B cell growth and differentiation, isotype switching to IgE, and eosinophil differentiation and activation – responses that may lead to protection against some parasites but that have also been implicated in allergy and hypersensitivity. The multiple factors controlling this critical divergence in the immune response include early cytokine production by other cell types, particularly dendritic cells. Thus, early IL-12 and IL-18 production in response to microbes or microbial products stimulates IFN- $\gamma$  production by T and NK cells and drives the response toward the Th1 pattern.<sup>142</sup> In addition, fungi have been shown to directly stimulate NK and other innate cells to secrete IL-10 or IFN- $\gamma$ <sup>143</sup> which may affect the nature of the Th response.

Studies in experimental animals suggest that protection against a number of fungi may be associated with a Th1-type response. In studies of murine systemic candidiasis, investigators have shown that the balance between Th1 and Th2 cytokines can be influenced by the *C. albicans* strain used for priming, the mouse strain, the route of initial inoculation (gastrointestinal colonization induced a Th1 pattern and intravenous injection a Th2 pattern), and the fungal morphotype. In vitro, ingestion of yeasts induced IL-12 release from DC and priming of Th1 cells, whereas ingestion of hyphae inhibited IL-12 and induced IL-4 production.<sup>123</sup> In vivo, generation of antifungal protective immunity was induced upon injection of DC pulsed with *Candida* yeasts but not hyphae. The immunization capacity of yeast-pulsed DC was lost in the absence of IL-12, whereas that of hypha-pulsed DC was gained in the absence of IL-4. Furthermore, a number of interventions that convert a Th2 to a Th1 response, such as administration of IFN- $\gamma$ , antibody to IL-4 or IL-10, or soluble IL-4 receptor, are also associated with subsequent protection.<sup>144-147</sup> Mice with self-limiting infections that were treated with antibody to IL-12 developed progressive disease associated with a Th2 response.<sup>148</sup> In summary, although neutrophils are of primary importance in protection from systemic candidiasis, considerable evidence suggests that in mice with normal phagocyte function, induction of a Th1-type cell-mediated response enhances clearance of the organism.



In the case of invasive aspergillosis, the murine data linking a Th1 pattern of cytokine production to resistance<sup>149</sup> is supplemented by clinical studies showing that healthy individuals and patients with aspergillosis responding to therapy had strong lymphoproliferative responses to *Aspergillus* antigen with a high IFN- $\gamma$ /IL-10 ratio, in contrast to patients whose infections were progressing.<sup>150</sup> In addition, serum IL-10 levels were high in non-neutropenic patients with aspergillosis and increased further in those failing therapy.<sup>151</sup> Similar to the observations in murine candidiasis, murine dendritic cells initiated a Th1 response when challenged with aspergillus conidia but a Th2 response when challenged with hyphae.<sup>121</sup>

Clinical and experimental animal studies also suggest a Th1-type response is associated with protection against *C. immitis* and *H. capsulatum*. In inbred mouse strains, resistance to *C. immitis* is correlated with a Th1-type response; susceptible mice can be rendered more resistant by treatment with IFN- $\gamma$ , anti-IL-4 or IL-12, and resistant mice can be made more susceptible by anti-IFN- $\gamma$  or anti-IL-12.<sup>152,153</sup> In patients with coccidioidomycosis, high complement-fixing antibody titers, more suggestive of a Th2-type response, are associated with a worse prognosis, whereas return of a delayed-type hypersensitivity response is a good prognostic sign.<sup>154</sup> Using peripheral blood mononuclear cells (PBMC) and whole-blood assays, IFN- $\gamma$ , but not IL-4 or IL-10, expression and release in response to coccidioidal antigen was higher in healthy immune donors than patients with acute disseminated disease, and was lowest in those with most severe infection.<sup>155-157</sup> An inverse correlation between cell-mediated immune responses and antibody titers is also found in patients with histoplasmosis. In murine histoplasmosis, resistance is associated with higher levels of IFN- $\gamma$  and susceptibility with early IL-4 induction. Furthermore, susceptible mice could be protected by administration of IL-12, an effect mediated by IFN- $\gamma$ .<sup>158</sup> Similar associations between Th1 patterns of response and protection have been described for *C. neoformans*, *B. dermatitidis* and *P. brasiliensis*.<sup>159-162</sup>

Notwithstanding the consistency of the above data, much remains to be learnt about the complexities of the development, maintenance, and regulation of Th1-mediated protection. In some systems, Th2 and regulatory cytokines such as IL-4 and IL-10 have nevertheless been shown to be necessary for immunity in models in which protection is in general Th1 mediated.<sup>163,164</sup> Other proinflammatory cytokines, especially IL-1 $\beta$ , TNF- $\alpha$  and IL-6, appear involved and important in the development of Th1-type responses. In murine histoplasmosis, absence of IL-1 signaling resulted in reduced resistance to primary infection that was associated with reduced IFN- $\gamma$  and increased IL-4 and IL-10 in the lungs,<sup>165</sup> while neutralization of TNF- $\alpha$  lead to reduced resistance to secondary infection, again associated with increased lung concentrations of IL-4 and IL-10.<sup>166</sup>

One possible mechanism contributing to the latter observation is the proapoptotic effect of TNF- $\alpha$ . Inhibition of apoptosis was found to increase IL-4 and IL-10 and increase susceptibility to histoplasmosis, suggesting that the lymphocytes that are normally eliminated by this means serve a regulatory function.<sup>167</sup> Reports of histoplasmosis, coccidioidomycosis, cryptococcosis, and candidiasis in patients treated with antibodies to TNF- $\alpha$  emphasize the importance of this cytokine in host defense against fungal infection.<sup>168</sup> In cryptococcosis, IL-6 has been associated with enhanced antifungal immunity in vitro

studies with human PBMC<sup>169</sup> and murine models.<sup>170</sup> IL-6 levels in the CSF of patients with cryptococcal meningitis were tightly correlated with levels of IFN- $\gamma$  and TNF- $\alpha$ , and high levels were associated with survival. In multivariate analysis, CSF levels of IFN- $\gamma$  were independently associated with the rate of clearance of infection from the CSF, confirming the key role of Th1-type immunity in protection against cryptococcosis in vivo in the human system.<sup>171</sup>

While Th1 responses appear critical for protection against invasive infections, it is possible that study of Th2 responses will shed light on the pathogenesis of some of the allergic manifestations of fungal exposure or colonization.<sup>172</sup>

## The role of CD8 T cells

CD4 Th cells are critical for a protective Th1-type immune response to fungal infection, but there is evidence that CD8 T cells may also play an important role. CD8 T cells recognize peptides complexed with MHC class I molecules. Such peptides are generated in the cytosol through the action of proteasomes and then transported into the endoplasmic reticulum where they associate with newly synthesized class I molecules before transport through the Golgi apparatus to the cell surface. DC have been shown to be capable of "cross-presentation" of exogenous antigens to CD8 T cells following phagocytosis of fungi.<sup>119,122</sup>

Romani and colleagues, using an intravenous model of systemic candidiasis, showed that CD4 T cells play the dominant role in the development of a protective response after priming with an avirulent *C. albicans* strain, but that both CD4 and CD8 cells are involved in the expression of resistance to a subsequent lethal challenge.<sup>173,174</sup> In murine cryptococcosis, CD8 cells have been shown to be involved in the development of a DTH response<sup>175</sup> and in resistance to pulmonary infection.<sup>176</sup> In murine histoplasmosis, although CD4 cells are critical for protection in immunocompetent mice and CD4 cells from immune mice can transfer protection to naive animals,<sup>177,178</sup> CD8 cells are also required for optimal elimination of the organism.<sup>179</sup> Furthermore, for histoplasmosis and blastomycosis, immunity can be induced by vaccination with *H. capsulatum* or *B. dermatitidis* yeasts in mice lacking CD4. The protection is MHC class I restricted, mediated by CD8 T cells, and associated with CD8 T cell-derived IFN- $\gamma$ , TNF- $\alpha$ , and granulocyte macrophage colony-stimulating factor (GM-CSF).<sup>180</sup> The results emphasize the redundancy of the immune response and have important implications for vaccination of CD4-deficient hosts such as those with HIV infection.

## Regulatory responses and suppression of the immune response

Recently, in addition to Th1 and Th2, other inducible CD4 T cell subsets have been described that secrete a predominance of regulatory cytokines, type 1 regulatory (Tr1) cells, secreting high levels of IL-10, Th3 cells secreting high levels of transforming growth factor- $\beta$  (TGF- $\beta$ ), and Th17 cells, eponymously named for their production of IL-17.<sup>181</sup> Thus, rather than a dichotomy between Th1 and Th2 responses, in some circumstances, a Th1 response associated with immunity to infection is balanced by responses which may turn out to be better defined as regulatory rather than Th2. Unregulated, immune responses

that limit microbial growth may also lead to immune-mediated pathology. The contribution of both microbial and immune effects to host damage is seen when the immune response is either too “weak” or too “strong,” respectively.<sup>1</sup> Many of the clinical manifestations of the mycoses, especially in immunocompetent hosts, result from the inflammatory response to fungal antigens. Thus, cytokines that have suppressive effects, including IL-10 and TGF- $\beta$ , when produced in appropriate amounts may be important in limiting damage to host tissues.

In addition to inducible, microbial antigen-specific CD4 regulatory T cells described above, much recent work has focused on natural regulatory CD4 T cells.<sup>182,183</sup> These mature in the thymus and exit functionally committed and characterized by expression of the IL-2 receptor, CD25, and the transcriptional factor Foxp3. The role of these cells in immunity to infection is under intense investigation but it appears they accumulate and proliferate at the site of infection and limit tissue damage, but also limit antimicrobial immunity, through the effects of IL-10, TGF- $\beta$  and cell-cell contacts. It may be that at least some natural T regulatory cells are specific for self antigens. Populations of CD4 CD25 T cells that reduce inflammation but may diminish antimicrobial immunity have been described in murine candidiasis and aspergillosis,<sup>184,185</sup> although at least in some instances these appear to be induced rather than natural. Much work remains to be done with regard to the role of these cell populations in immunity to fungal infections. Paradoxically, TGF- $\beta$ , which is involved in the differentiation and function of regulatory T cells, is also involved, with IL-6 and IL-23, in driving the development of Th17 cells, that through secretion of IL-17 cytokines have proinflammatory and neutrophil-mobilizing effects.<sup>141,186</sup> Stimulation of DC via dectin-1 biases towards Th17-type responses.<sup>187</sup>

Co-evolution of host and microbes may lead to immune regulation that limits tissue damage and allows low-level persistence of infection that is necessary to maintain protection from re-challenge. On the other hand, excessive regulation, in some instances triggered by microbial products, may be detrimental to the host and a means by which pathogens evade elimination. For example, cryptococcal glucuronoxylomanan has been found to have numerous suppressive effects on innate and adaptive immunity.<sup>67</sup> It is bound by multiple pattern recognition receptors on innate cells, including TLR 4 and 2 and the inhibitory Fc gamma receptor II,<sup>188</sup> and causes IL-10 release from macrophages. The latter may be important in favoring the development of a regulatory or Th2 over Th1 pattern of T cell cytokine response. In relation to *C. albicans*, mannose-containing oligosaccharides have been shown to non-specifically inhibit lymphoproliferative responses to antigen.<sup>189</sup> Such oligosaccharides derived from the breakdown of *Candida* cell wall mannan in vivo could contribute to the depression of cell-mediated immunity seen in chronic candidiasis. Examples of other immunomodulatory fungal products include prostaglandins produced by *C. neoformans* and *C. albicans*,<sup>190</sup> and the mycotoxin gliotoxin of *A. fumigatus*.<sup>191</sup>

## Recruitment of leukocytes and organization at the site of infection

For those fungi for which an adaptive cell-mediated immune response is critical in protection, mononuclear phagocytes and lymphocytes play a key role in the further recruitment

of immune cells into the site of infection and in containment of the infection through granuloma formation. Huffnagle and colleagues found that clearance of less virulent strains of *C. neoformans* after intratracheal infection in mice was associated with a relatively early and large influx of macrophages into the lungs.<sup>192</sup> This recruitment depends on early production of proinflammatory cytokines such as TNF- $\alpha$ ,<sup>193</sup> perhaps by alveolar macrophages, chemokines, and the subsequent development of specific T cell immunity, and in particular CD4 cells. TNF- $\alpha$  enhances T cell proliferation and cytokine production, and is required for the development of T cell-mediated immunity in this model. TNF- $\alpha$  depletion prevents induction of IL-12 and IFN- $\gamma$ ,<sup>194</sup> an effect that may be related to its role in the maturation and accumulation of DC in lung lymph nodes.<sup>195</sup> Leukocyte recruitment is also dependent on the production of chemokines, such as MCP-1 and MIP-1 $\alpha$  (members of the C-C family of chemokines that are chemotactic predominantly for mononuclear cells) by macrophages, T cells, and non-leukocyte cells, and the expression of endothelial adhesion molecules that mediate leukocyte binding and diapedesis.<sup>196,197</sup> Mice deficient in the MIP-1 $\alpha$  ligand, CCR-5, have a specific defect in recruitment to the CNS and protection against CNS infection.<sup>198</sup> T cells are also required for monocyte/macrophage recruitment such that, in their absence, the inflammatory response is delayed and composed mainly of neutrophils.<sup>199</sup> After recruitment, CD4 T cells are required for the formation of granulomas and the confinement of *C. neoformans* within multinucleated giant cells.<sup>200</sup> Finally, as noted above, complement activation results in the generation of the potent leukocyte chemotaxin, C5a.

For the fungal infections for which Th1 immunity is paramount, protection is associated with a well-defined granulomatous organization of immune cells in the tissues. A few studies have attempted to characterize the immunologic organization of granulomas from patients with fungal disease. Coccidioid pulmonary granulomata were found to comprise central necrosis with a mantle of roughly equal numbers of CD4 and CD8 T lymphocytes, within which there were distinct clusters comprising roughly equal numbers of T and B cells.<sup>201</sup> IFN- $\gamma$  was expressed by a third of cells in the mantle but by very few cells in the clusters, and IL-10 by 40% and 24% of cells in the mantle and clusters, respectively. IL-10 was expressed by B and CD4 T cells, but not CD8 T cells.

## Activation of effector cells

There are several possible mechanisms by which activated T cells could mediate protection. Probably the most important is through the secretion of cytokines that enhance the antifungal activity of other effector cells such as macrophages, neutrophils, and NK cells. In addition, however, activated T cells may themselves have antifungal activity,<sup>202</sup> and CD8 cells could lyse infected macrophages,<sup>119</sup> releasing fungal cells to be ingested by more potently activated cells. Both intracellular and extracellular inhibition or killing may be involved.

For those fungi controlled by cell-mediated immune responses, mononuclear phagocytes may be the most important final effector cells that clear the organism. As discussed above, macrophages from different species and different anatomic sites vary in their capacity to inhibit and kill fungi. Moreover, murine and human macrophages may require different signals to become activated to kill fungi. IFN- $\gamma$  has been shown to increase

the activity of murine macrophages against a number of fungi, including *C. neoformans*, *H. capsulatum*, *C. albicans*, *B. dermatitidis* and *C. immitis*, by mechanisms including nitric oxide generation and restriction of iron availability.<sup>203,204</sup> In contrast to studies with murine cells, it has been less easy to demonstrate antifungal activity for activated human macrophages. Although IFN- $\gamma$  has been reported to increase the activity of human monocyte-derived macrophages against *C. albicans*,<sup>205</sup> most studies have found that IFN- $\gamma$  does not enhance the activity of human macrophages against *C. neoformans* or *H. capsulatum*.<sup>206-209</sup> Generation of microbicidal concentrations of nitric oxide has been difficult to demonstrate in human macrophages in vitro, which may help to explain why the effects of IFN- $\gamma$  seen in mouse macrophages have not always been reproduced in human cells.

A number of other activating factors and fungicidal mechanisms may be involved in human macrophages. Human monocyte-derived macrophages did limit the growth of *H. capsulatum* if the colony-stimulating factors (CSF) IL-3, granulocyte macrophage (GM)-CSF or macrophage (M)-CSF were present during the process of differentiation.<sup>209</sup> GM-CSF and IL-3 were also shown to enhance the activity of human monocytes and monocyte-derived macrophages against *C. albicans*.<sup>210</sup> In contrast, a variety of cytokines (IFN- $\gamma$ , TNF- $\alpha$ , IFN- $\gamma$  + TNF- $\alpha$ , GM-CSF) were found not to enhance the anticryptococcal activity of human monocyte-derived macrophages.<sup>206</sup>

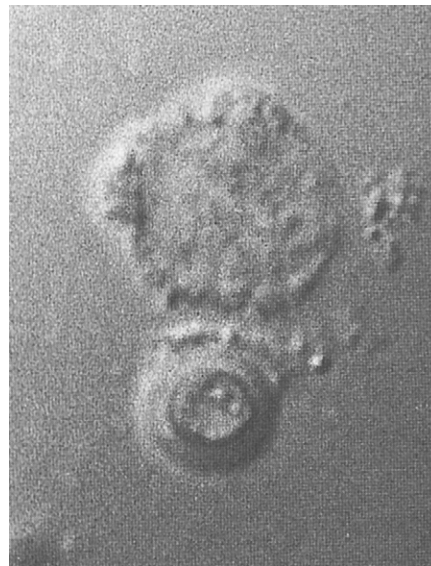
Activated macrophages are important effector cells, but cytokines generated in the course of a specific cell-mediated immune response may also enhance the antifungal activity of NK cells, cytotoxic CD8 and CD4 T lymphocytes, and neutrophils. For example, although NK cells have constitutive anticryptococcal activity, IL-12 was shown to enhance the activity of purified NK cells from HIV-infected donors against *C. neoformans*.<sup>211</sup> IL-2 has been shown to increase the activity of murine CD8 cells against *C. albicans*,<sup>212</sup> and IL-2 and IL-15 to enhance the anticryptococcal activity of CD4 and CD8 T cells, respectively, from normal donors.<sup>213-215</sup> TNF- $\alpha$ , IFN- $\gamma$ , IL-8, G-CSF, GM-CSF, and IL-2 have all been shown to increase the activity of neutrophils against *C. albicans* blastoconidia.<sup>216-218</sup> G-CSF and IFN- $\gamma$ , but not TNF- $\alpha$ , were also shown to enhance neutrophil killing of *C. albicans* hyphae.<sup>105,219</sup>

## Natural killer (NK) and cytotoxic t cells

NK cells are a subset of large lymphocytes with numerous cytoplasmic granules with the ability to selectively lyse certain tumor and virally infected cells.<sup>220</sup> NK cells lack the somatically recombined antigen receptors of T and B cells, but do possess receptors that recognize class I MHC molecules in association with self peptides. Such recognition switches off cytolytic mechanisms and so protects normal host cells.<sup>221</sup> NK cells also have Fc $\gamma$  III (CD16) receptors and can lyse target cells coated with IgG (antibody-dependent, cell-mediated cytotoxicity). The balance of inhibitory and stimulatory signals through a range of NK receptor-ligand pairs determines NK cell activation. Killing of target cells may involve granule exocytosis with release of perforin (which is homologous to C9 and forms pores in the membranes of target cells), serine esterases (also called granzymes), and other enzymes. Apoptosis (programmed

cell death) can result because of involvement of Fas ligand on the effector cells triggering a Fas-mediated pathway in the target cells.<sup>222</sup> The cytolytic activity of NK cells is enhanced by IFN- $\gamma$ , IL-12, and IL-2. Cells stimulated with high concentrations of IL-2 lose some target specificity and have been called lymphokine-activated killer (LAK) cells. Activated NK cells also secrete cytokines, in particular IFN- $\gamma$ , which could activate macrophages before the development of a specific T cell-mediated response. Moreover, interactions between NK cells and DC in tissues and lymph nodes may play an important role in shaping the adaptive immune response.<sup>223</sup>

With regard to host defense against fungi, NK cells have been shown to bind to and inhibit the growth of *C. neoformans*,<sup>224</sup> *P. brasiliensis*,<sup>225</sup> and *C. immitis*<sup>226</sup> in vitro. Murphy and colleagues<sup>227</sup> demonstrated killing of *C. neoformans* by murine NK cells and some growth inhibition of *C. neoformans* by cytoplasmic granule fractions and perforin purified from granule fractions from rat NK tumor cells.<sup>228</sup> The involvement of granule exocytosis in NK cell-mediated growth inhibition of *C. neoformans* is also suggested by imaging human NK cell-*C. neoformans* conjugates in which the granules seem to be in the process of being discharged on the fungal surface (Fig. 3-4).<sup>229</sup> Mody and colleagues recently demonstrated that the constitutive anticryptococcal activity of human NK cells is mediated by perforin.<sup>230</sup> In addition to their direct antifungal activity, NK cells could play a role in host defense through production of cytokines. Although growth inhibition of *C. albicans* by human NK cells has not been demonstrated, human NK cells bind *C. albicans*, causing release of cytokines, including GM-CSF, TNF- $\alpha$  and IFN- $\gamma$ , that could activate both neutrophil and mononuclear phagocyte effector cells, and favor development of a Th1 response.<sup>143,231</sup>



**Figure 3-4** Nomarski microscopy demonstrating binding of a human NK cell to *C. neoformans*. The fungal cell is easily identified by its thick cell wall. Granules from the NK cell can be seen concentrated next to the fungal cell and appear to be in the process of being discharged onto the fungal surface (reproduced with permission from Levitz et al.<sup>229</sup>).

However, *in vivo* studies have suggested a limited role for NK cells in host defense against fungi. For *C. neoformans*, NK cells may contribute toward early clearance of the organism from the lung. When mice were depleted of NK cells and then challenged with *C. neoformans* intravenously, yeast colony-forming units were increased in the lungs at early time points compared with control mice. However, no differences were seen later in infection, in other organs or following intratracheal challenge.<sup>232,233</sup> In similar experiments in mice infected with *H. capsulatum* and *C. albicans*, no significant role for NK cells in host defense against these fungi could be demonstrated.<sup>234-236</sup>

T lymphocytes have also been shown to directly bind to and inhibit the growth of *C. neoformans* and *C. albicans* *in vitro*.<sup>213,229,237</sup> Against *C. neoformans*, both human CD4 and CD8 cells were found to have activity, which could be enhanced by culture with IL-2 or phytohemagglutinin. Lymphocytes were observed to form reversible conjugates with cryptococci with broad areas of contact between the lymphocyte membrane and the fungal capsule. The receptor(s) and ligand(s) responsible for this attachment remain uncertain. However, opsonization of the fungus is not required. CD8 T cell activity is dependent on granulysin,<sup>214</sup> a lipid-binding protein present in the granules of cytotoxic lymphocytes that has direct activity against *C. neoformans*.<sup>238</sup> CD8 T cell activity required CD4 T cell help, through activation of accessory mononuclear cells, to produce IL-15. CD4 T cells also use granulysin to inhibit *C. neoformans*. Expression of granulysin in CD4 T cells required 5–7 days stimulation with IL-2, and was defective in CD4 T cells from HIV-infected patients, even at an early stage of disease (CD4 T cell counts >400/μl).<sup>215</sup> Similarly, IL-2-stimulated murine CD8 lymphocytes have been shown to bind to and inhibit the growth of *C. albicans* hyphae.<sup>212</sup> Adhesion to hyphae depends on the participation of lymphocyte CR3 (CD11b/CD18) receptors.<sup>239</sup> The importance *in vivo* of this direct antifungal activity of non-specific T cells has yet to be determined. It is also possible that non-specific CD8 T cells, like NK cells, play a role in innate immunity through IFN-γ secretion, as has been demonstrated in the context of intracellular bacterial infection.<sup>240</sup>

## Effects of HIV on the immune response to fungi

HIV-1 infection is a major risk factor predisposing people to serious fungal infections. In particular, mycoses due to *C. neoformans*, *C. albicans*, *Pneumocystis jirovecii* and, in endemic regions, *H. capsulatum*, *C. immitis*, and *Penicillium marneffei* are greatly increased in prevalence in persons with HIV infection, especially as the disease progresses to AIDS. Aspergillosis is also seen with increased frequency in those with late-stage AIDS.<sup>241</sup> Patients infected with HIV contract many immunologic abnormalities,<sup>242</sup> but the specific mechanisms underlying the susceptibility to opportunistic mycoses seen in individuals with late-stage HIV disease are still incompletely understood. Certainly the profound and progressive CD4+ T cell depletion that is the immunologic hallmark of AIDS is the major contributor. Indeed, for all the preceding mycoses there is a strong inverse correlation between CD4+ T cell count and risk of infection.<sup>243</sup> Moreover, the critical role of CD4 cells in host

defenses is supported by the frequent finding of disseminated fungal infections in patients with idiopathic CD4 T lymphocytopenia, an entity characterized by low CD4 T cell counts in the absence of HIV infection.<sup>244</sup>

HIV infection also has effects on the immune system that are independent of CD4 T cell depletion. HIV directly infects monocytes and macrophages and mononuclear phagocytes from HIV-infected individuals have been shown to have alterations in phenotypic marker expression, chemotaxis, cytokine production, and respiratory burst activity.<sup>245,246</sup> Interestingly, mononuclear phagocyte dysfunction can be demonstrated relatively early in HIV infection, before CD4+ T cell depletion has progressed. This dysfunction may result from the direct effects of HIV infection, from soluble products released by HIV, in particular the envelope glycoprotein gp120, or indirectly from alterations in the cytokine milieu. Conflicting results have been reported when the functional consequences of *in vitro* infection of healthy monocytes with HIV have been examined. Cameron et al<sup>247</sup> inoculated human blood monocytes, peritoneal macrophages, and bronchoalveolar macrophages with a monocytotropic strain of HIV. Monocytes and peritoneal macrophages had a transient reduction in anticytotoxic activity that correlated with a period of maximal viral replication. In contrast, no effects were seen in bronchoalveolar macrophages. Addition of gp120 to normal human bronchoalveolar macrophages resulted in inhibition of anticytotoxic activity.<sup>248</sup> Although gp120 did not affect cryptococcal binding, it did inhibit the subsequent internalization of bound yeasts.

Peripheral blood leukocytes from HIV-infected individuals have been shown to have impairments in aspects of both the afferent and efferent arms of the cell-mediated response to fungal pathogens. PBMC from HIV-infected donors have profoundly impaired proliferative responses to fungal antigens as to other recall antigens.<sup>249-252</sup> The pattern of cytokine production is also altered: for example, in response to *C. neoformans*, *C. albicans*, and *C. immitis* antigen, expression and release of IFN-γ by PBMC from HIV-infected donors is markedly reduced.<sup>253,254</sup> There is also a deficit in IL-12 production that can be restored by priming with IFN-γ before stimulation.<sup>253</sup> Monocytes from HIV-infected donors had reduced anticytotoxic activity, respiratory burst, and degranulation compared with that for control monocytes.<sup>255,256</sup> Defects in binding and growth inhibition of *H. capsulatum* were also shown in cultured monocytes from HIV-infected persons.<sup>257</sup> Similarly, neutrophils from AIDS patients have impaired the fungicidal activity against *C. albicans* and *C. neoformans* compared with control subjects,<sup>258</sup> and NK and CD4 T cells from HIV-infected donors have impaired anticytotoxic activity.<sup>211,215</sup> *In vivo* administration of recombinant human G-CSF enhanced *in vitro* neutrophil fungicidal activity and augmented the respiratory burst. *In vitro*, IL-12 restored the anticytotoxic activity of NK cells from HIV-infected donors.<sup>211</sup> Combination antiretroviral therapy has now been documented to reverse some of these *ex vivo* abnormalities.<sup>259</sup>

In addition to these effects of HIV on the immune response to fungi, stimulation of HIV-infected T cells and mononuclear phagocytes *in vitro* with fungi or fungal products induces HIV replication.<sup>260-262</sup> This finding may have clinical implications, because induction could increase the viral load, accelerating the course of HIV disease and further impairing host defense,



systemically and locally at the site of infection, against the fungal pathogen.

The above in vitro and ex vivo studies have been complemented by data on the in vivo immune response in HIV-infected individuals, taking advantage of the fact that in cryptococcal meningitis, sampling at the site of infection in the CSF is possible. Lortholary and colleagues found that the proinflammatory cytokines IL-6, IL-8, and TNF- $\alpha$ , as well as IL-10, were all lower in the CSF of HIV-infected compared to non-infected patients with cryptococcal meningitis.<sup>263</sup> In a separate study of HIV-infected cryptococcal patients, the trio of CSF IL-6, TNF- $\alpha$  and IFN- $\gamma$  was highly correlated, higher levels were associated with survival, and IFN- $\gamma$  was shown to be an independent factor predicting the rate of clearance of infection.<sup>171</sup>

In addition, it has become clear that restoration of immunity following antiretroviral therapy not infrequently leads to exacerbation of clinical disease in patients with fungal, as well as other, opportunistic infections.<sup>264,265</sup> Pathology specimens, if available, are liable to show increased granulomatous inflammation compared to patients prior to antiretroviral therapy. In patients with cryptococcal immune reconstitution inflammatory syndrome (IRIS), the CSF white cell count is often higher than in patients presenting with cryptococcal meningitis prior to antiretroviral therapy,<sup>266</sup> but the further immunologic correlates of this enhanced response are still under investigation.

## Vaccination and immunotherapy

The development of vaccines against the important systemic fungal infections is under intense investigation.<sup>267</sup> A vaccine consisting of formaldehyde-killed spherules of *C. immitis* was protective in a mouse model of coccidioidomycosis, but in a large trial of susceptible persons in the endemic area, efficacy could not be demonstrated and local reactions limited the dose that could be given.<sup>268</sup> Immunization with subcellular fractions or purified antigens is likely to be better tolerated. A number of studies have identified fungal antigens that are important targets of B- and T-cell responses; varying degrees of protection were seen when these vaccine candidates were tested in animal models of mycoses.<sup>267,269-271</sup> Most serious fungal infections occur in significantly immunocompromised patients who are likely to have a suboptimal response to immunization. Thus, for any vaccine, it will be a formidable challenge to demonstrate that immunization can confer protection for the patient population at risk.

An appealing strategy is to identify antigens that are conserved amongst pathogenic fungi in the hope that a vaccine that elicits protection against a broad range of fungal pathogens can be developed. Mice immunized with a vaccine composed of a  $\beta$ -1,3-glucan conjugated to diphtheria toxoid developed a protective IgG immune response against lethal infections of *C. albicans* and *A. fumigatus*.<sup>272</sup> Passive therapy has also been attempted. Mycograb® is a recombinant antifungal human monoclonal antibody that binds to HSP90, a highly conserved cellular chaperone found in *Candida* species. Mycograb®, in combination with amphotericin B, was superior to amphotericin B alone in a multicenter, double-blind, placebo-controlled trial of patients with invasive candidiasis.<sup>273</sup> Passive immunotherapy using a monoclonal antibody against the major *C. neoformans*

capsular polysaccharide, glucuronoxylomannan, was evaluated in a phase 1 dose escalation study of patients with cryptococcosis.<sup>274</sup> Modest, transient reductions in antigen titers were seen. Active immunization to elicit antibodies against glucuronoxylomannan to protect high-risk patients from developing cryptococcosis has been studied in murine models of cryptococcosis.<sup>270,275</sup>

Successful vaccines designed to boost T cell-mediated immunity will need to take into account the genetic heterogeneity of the human population, particularly the capacity to respond to T cell epitopes. Thus, while in murine models of mycoses individual antigens can sometimes be protective, vaccines that incorporate multiple antigens will likely be needed for humans.<sup>267</sup> Strategies to elicit strong Th1-type responses have included using CpG as an adjuvant and administering antigen- or RNA-pulsed DC.<sup>267,276,277</sup> In experiments utilizing cells from humans with coccidioidomycosis, reversal of T cell anergy was achieved with human DC pulsed with fungal antigens.<sup>278</sup> Strategies involving ex vivo expansion and infusion of specific T cells, as used for post bone marrow transplantation EBV and CMV infections, are also under development for aspergillosis.<sup>279</sup> A "therapeutic" vaccine to boost cell-mediated immune responses has been tested in humans and horses infected with the fungus-like organism *Pythium insidiosum*.<sup>280,281</sup> Administration of the vaccine, composed of crude antigens, resulted in disease remission in 50% of the infected patients.

A strong rationale exists for adjunctive immunotherapy with IFN- $\gamma$  in selected systemic fungal infections, given the evidence demonstrating a key role for Th1 responses in host defense. In HIV-associated cryptococcal meningitis, a randomized, placebo-controlled trial of IFN- $\gamma$  given in addition to amphotericin B showed a trend toward benefit in terms of the proportion of patients with a sterile CSF culture after 2 weeks of treatment.<sup>282</sup> Adjunctive IFN- $\gamma$  also appeared to be safe. Further studies with more powerful endpoints are warranted. In addition, although the evidence is largely anecdotal, IFN- $\gamma$  and colony-stimulating factors have also been used in small numbers of cancer patients with refractory fungal infection, with apparently beneficial effects.<sup>283</sup>

## Conclusion

As the world's immunodeficient population grows as a result of the HIV pandemic and increased use of highly immune suppressive regimens to treat a variety of illnesses, fungal infections will continue to be a major clinical problem.<sup>116</sup> Moreover, mycoses are predicted to be associated with many of the newer immunosuppressive medications that are making their way into clinical use, such as has already been observed with the anti-TNF- $\alpha$  therapies.<sup>284</sup> While remarkable advances have been made in understanding how the immune system responds to fungal pathogens, many important questions remain unanswered. While most patients with systemic mycoses have an identifiable predisposing immunocompromise, some do not. Additionally, even amongst those with predisposing risks, attack rates and severity of disease can vary substantially. New molecular tools to study immunogenetics should help to better define some of the more subtle predisposing factors that might exist, such as single nucleotide polymorphisms in immune



response genes. Immunogenetic analyses could also unravel the mystery of why susceptibility to coccidioidomycosis varies amongst racial and ethnic groups.

An in-depth understanding of the host immune response to fungal pathogens is important when choosing antifungal therapies and in the rational application of new therapeutic modalities.

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# The laboratory and clinical mycology

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## Introduction

Serious infections are being reported with an ever-increasing array of pathogens (Table 4-1). It is now clear that there are no non-pathogenic fungi; virtually any fungus can cause a lethal mycosis in an immunocompromised host. It is absolutely essential that institutions caring for high-risk immunocompromised patients place a high priority on maximizing their diagnostic capabilities for the early detection of opportunistic fungal infections. Successful diagnosis and management of such infections in the compromised patient are highly dependent on a team approach involving clinicians, microbiologists, and pathologists (Table 4-2).

## Laboratory diagnosis

### Specimen collection and processing

Selection of appropriate specimens for culture and microscopic examination is based on the results of clinical and radiographic examination and consideration of the most likely fungal pathogen that may cause such an infection (Tables 4-3, 4-4). Specimens should be collected under aseptic conditions or after appropriate cleaning and decontamination of the collection site and rapidly transported to the laboratory. Unfortunately, many specimens submitted to the laboratory are either of insufficient amount or of poor quality and are inadequate to make a diagnosis.

The clinical information is very important in guiding the laboratory efforts in terms of specimen processing and interpretation of results. This is especially important when dealing with specimens from non-sterile sites such as sputum, bronchial washings and skin. Furthermore, it is the only way of effectively alerting the laboratory personnel that they may be dealing with a potentially dangerous pathogen such as *Histoplasma capsulatum* or the *Coccidioides immitis* complex.<sup>1</sup>

Most fungi can be recovered from specimens submitted in bacteriologic transport media, although direct microscopic examination of such material is not recommended because the transport medium components can hinder the observation of the fungi. In general, if a delay in processing is unavoidable,

the specimen for the fungal culture may be safely stored at 4°C for a short time.

Some specimens are better than others for the diagnosis of fungal infections (see Table 4-4). Swab specimens are inappropriate for mycologic culture and microscopic examination. Cultures of blood, cerebrospinal fluid (CSF) and other normally sterile body fluids (NSBF) should be performed if clinical signs and symptoms are suggestive of hematogenous dissemination or involvement of these sites. Diagnosis of oral or vaginal mucosal infections may be better established by clinical presentation and direct microscopic examination of secretions or mucosal scrapings. Likewise, diagnosis of fungal infections of the gastrointestinal tract is better established by biopsy and histopathologic examination of involved tissue than by culture alone. Care should be taken in collecting lower respiratory and urine specimens to minimize contamination with normal oral and periurethral flora, respectively. Twenty-four hour collections of sputum or urine are inappropriate for mycologic examination because they typically become overgrown with both bacterial and fungal contaminants.

### Stains and direct examination

Direct microscopy does not utilize fixed tissue and relies instead on rapid examination of wet mounts (Table 4-5). Often infections are caused by organisms that can be specifically identified by direct microscopy because they possess a distinctive morphology. For example, if typical yeast cells, spherules or other structures are observed microscopically, an etiologic diagnosis can be made for infections caused by *H. capsulatum* (Fig. 4-1), *Blastomyces dermatitidis* (Fig. 4-2), *Cryptococcus neoformans* (Fig. 4-3), *C. immitis* complex (Fig. 4-4), and *Pneumocystis jiroveci* (syn. *P. carinii*) (Fig. 4-5). In other infections such as aspergillosis (Fig. 4-6), candidiasis (Fig. 4-7), and zygomycosis (Fig. 4-8), the morphologic appearance may lead to a diagnosis of the type of infection but not the actual species identification of the etiologic agent (Table 4-6).

Detection of fungi in tissue and clinical material by direct microscopic examination is often helpful in determining the significance of culture results. Detection of specific fungal elements by microscopy can assist the laboratory in selecting the most appropriate means by which to culture the clinical

**Table 4-1** Spectrum of opportunistic fungal pathogens<sup>a</sup>

Organisms group	Examples of specific pathogens	
<i>Candida</i>	<i>C. albicans</i> <i>C. glabrata</i> <i>C. parapsilosis</i> <i>C. tropicalis</i>	<i>C. krusei</i> <i>C. lusitaniae</i> <i>C. guilliermondii</i> <i>C. rugosa</i>
Other yeasts	<i>Cryptococcus neoformans</i> <i>Trichosporon</i> <i>Blastoschizomyces</i> <i>Rhodotorula</i> <i>Malassezia</i>	<i>Saccharomyces</i> <i>Hansenula</i>
<i>Aspergillus</i>	<i>A. fumigatus</i> <i>A. flavus</i> <i>A. niger</i>	<i>A. versicolor</i> <i>A. terreus</i> <i>A. nidulans</i>
Zygomycetes	<i>Rhizopus</i> <i>Rhizomucor</i> <i>Mucor</i> <i>Absidia</i>	<i>Apophysomyces</i> <i>Cunninghamella</i> <i>Saksenaia</i> <i>Cokeromyces</i>
Other hyaline moulds	<i>Fusarium</i> <i>Acremonium</i> <i>Scedosporium apiospermum</i> <i>S. prolificans</i>	<i>Trichoderma</i> <i>Paecilomyces</i> <i>Chrysosporium</i>
Dematiaceous moulds	<i>Alternaria</i> <i>Bipolaris</i> <i>Curvularia</i> <i>Exophiala</i>	<i>Cladophiala</i> <i>phora</i> <i>Phialophora</i> <i>Dactylaria</i> <i>Wangiella</i>
Dimorphic moulds	<i>Histoplasma</i> <i>Coccidioides</i> <i>Blastomyces</i> <i>Paracoccidioides</i>	<i>Sporothrix</i> <i>Penicillium marneffeii</i>
Other	<i>Pneumocystis jiroveci</i>	

<sup>a</sup>List not exhaustive.

specimen. For example, the presence of hyphae of a zygomycetous organism (see Fig. 4-8) should prompt the use of malt agar or even sterile bread without preservatives for its isolation.

A number of different stains and techniques may be used to help demonstrate the presence of fungi by direct microscopic examination (see Table 4-5). Most commonly, microscopy consists of examination of clinical material placed in 10–20% potassium hydroxide (KOH) containing the fluorescent reagent Calcofluor white (Fig. 4-9) or staining of individual smears or touch preparations by Gram, Giemsa, periodic acid-Schiff (PAS) or any combination of these stains. The Gram stain is useful for

**Table 4-2** Laboratory diagnosis of invasive fungal infections

A. Conventional microbiologic
1. Direct microscopy (Gram, Giemsa, and Calcofluor stains)
2. Culture
3. Identification
4. Susceptibility testing
B. Histopathologic
1. Conventional microscopy <ol style="list-style-type: none"> <li>Routine stains (H&amp;E)</li> <li>Special stains (GMS, Mucicarmine, PAS)</li> </ol>
2. Direct immunofluorescence
3. In situ hybridization
C. Immunologic
1. Cryptococcal antigen test
2. <i>Histoplasma</i> antigen test
3. Galactomannan test
4. Mannan test
D. Molecular
1. Direct detection
2. Identification
3. Strain typing
E. Biochemical
1. Metabolites (D-arabinitol)
2. Cell wall components ( $\beta$ -glucan)
Abbreviations: H&E, hematoxylin and eosin; GMS, Gomori's methenamine silver; PAS, periodic acid-Schiff.

the detection of *Candida* and *Cryptococcus* spp. (Figs 4-7, 4-10) and also stains the hyphal elements of moulds such as *Aspergillus* (Fig. 4-11), the zygomycetes (see Fig. 4-8), and *Fusarium* spp. Fungi are typically Gram positive but may appear speckled or Gram negative (Figs 4-7, 4-10). The capsular material of *C. neoformans* often appears as an orange-red precipitate around the cells (see Fig. 4-10). Many fungi will stain blue with the Giemsa stain but this stain is especially useful in detecting *H. capsulatum* intracellularly in bone marrow, peripheral blood, bronchoalveolar lavage (BAL) specimens, or touch preparations of lymph nodes or other tissues (see Fig. 4-1).

The morphologic characteristics of fungi seen on direct microscopic examination include budding yeasts, hyphae, and pseudohyphae (see Table 4-6). *Aspergillus* spp. typically show hyaline, dichotomous, acute angle branching, septate hyphae (Figs 4-11, 4-12); however, this appearance is also typical of other hyaline moulds (see Table 4-6). In contrast, zygomycetes (e.g., *Rhizopus*, *Mucor*) characteristically show broad, ribbon-like, aseptate or sparsely septate hyphae (see Fig. 4-9). Finally, the dematiaceous fungi often present as darkly pigmented yeast-like and hyphal forms that may be visualized on unstained material and further characterized by the Fontana-Masson stain for melanin (see Tables 4-5, 4-6).



**Table 4-3** Relative frequency of opportunistic mycoses among different patient groups<sup>a</sup>

Patient group <sup>b</sup>	Mycosis <sup>c</sup>											
	Asp	Can	Cryp	Tri	PCP	Hyal	Phae.	Blas <sup>d</sup>	Hist <sup>d</sup>	Cocci <sup>d</sup>	Pmar <sup>d</sup>	Zygo
<b>Transplant</b>												
Allo. BMT	++++	++	++	++	+++	++	+	(+)	(+)	(+)	(+)	++
Liver	+++	++++	+++	+	+	+	+	(+)	(+)	(+)	(+)	+
Lung	++++	+++	++	+	+	+	+	(+)	(+)	(+)	(+)	+
Kidney	++	+++	++	+	+	+	+	(+)	(+)	(+)	(+)	+
Heart	++++	+++	++	+	+	+	+	(+)	(+)	(+)	(+)	+
Pancreas	++	++++	+	+	+	+	+	(+)	(+)	(+)	(+)	+
Sm. bowel	++	++++	+	+	+	+	+	(+)	(+)	(+)	(+)	+
<b>Malignancy</b>												
Heme	+++	++++	++	+	++	+	+	(+)	(+)	(+)	(+)	+
Solid	++	++++	++	+	++	+	+	(+)	(+)	(+)	(+)	+
<b>HIV/AIDS</b>	++	++	+++		++++	+	+	(++)	(++++)	(++++)	(++++)	+
<b>Critical care</b>												
Adult	+	++++		+	+	+						+
Neonate		++++		+	+							+

<sup>a</sup>Relative frequency of mycoses within each patient group indicated as ++++ (most frequent) to + (least frequent). Adapted from Pfaller et al.<sup>2</sup>

<sup>b</sup>Patient group abbreviations: Allo, BMT, allogeneic blood and marrow transplant; Sm. bowel, small bowel; Heme, hematologic malignancy; Solid, solid tumor malignancy.

<sup>c</sup>Mycosis abbreviations: Asp, aspergillosis; Can, candidiasis; Tri, trichosporonosis; Cryp, cryptococcosis; PCP, *Pneumocystis jiroveci* (carinii) pneumonia; Hyal, hyalohyphomycosis; Phae, phaeohyphomycosis; Blas, blastomycosis; Hist, histoplasmosis; Cocci, coccidioidomycosis; Pmar, *Penicillium marneffei*; Zygo, zygomycosis. See Table 4-1 for specific examples within each group.

<sup>d</sup>Frequency of endemic mycoses indicated by (+) to (++++), within endemic regions only.

The laboratory diagnosis of a *P. jiroveci* infection is commonly made by direct examination of induced sputum and specimens collected by bronchoscopy. In addition to the more general stains such as Gomori's methenamine silver stain (GMS) (Fig. 4-13), Giemsa (see Fig. 4-5), and toluidine blue (see Table 4-5), the commercial availability of fluorescent monoclonal antibody-based conjugates has enhanced the detection of this organism and these conjugates provide a sensitive and highly specific diagnosis.<sup>3</sup>

## Culture

The most sensitive means of diagnosing a fungal infection is generally considered to be the isolation of the infecting agent on culture media. Having said this, false-negative cultures are well documented in the face of disseminated fungal infection and even when positive, the results may be delayed or difficult to interpret.<sup>4-7</sup> In most instances culture is necessary to specifically identify the etiologic agent and, if indicated, to determine the in vitro susceptibility to various antifungal agents.

Although not all serious fungal infections are marked by hematogenous dissemination and fungemia, detection of fungemia is useful in diagnosing opportunistic infection due to *Candida* spp., *C. neoformans*, *Trichosporon* spp., *Malassezia* spp., *Fusarium* spp., and occasionally *Acremonium* spp., *Paecilomyces* spp., *Scedosporium* spp., and *Aspergillus terreus*.<sup>8</sup> Blood cultures may be negative in the face of disseminated disease; however, advances in blood culture technology have markedly improved the ability of laboratories to detect fungemia.<sup>8,9</sup> It has been proposed that optimal detection of fungemia requires the collection of adequate volumes of blood (20–30 ml) and the use of both a broth- (vented, agitated) and an agar-based (lysis centrifugation) blood culture method.<sup>8</sup>

Interpretation of the results of fungal cultures may be difficult due to the frequent colonization of certain body sites (e.g., respiratory, gastrointestinal, and genitourinary tracts) and contamination of specimens or cultures by environmental organisms, many of which can also serve as etiologic agents of opportunistic mycoses. Whereas most isolates of *Candida* spp., *C. neoformans*, *H. capsulatum*, and *Fusarium* spp. obtained

**Table 4-4** Selection of clinical specimens for detection and isolation of opportunistic fungal pathogens<sup>a</sup>

Suspected pathogen	Blood	Bone marrow	Brain and cerebrospinal fluid	Joint fluid	Eye	Urine	Respiratory	Skin and mucous membranes	Multiple systemic sites
<b>Yeasts</b>									
<i>Candida</i> spp.	++++	+	++	+	+	+++	+	+++	+++
<i>Cryptococcus neoformans</i>	+++	+	++++		+	++	+++	+	++
<i>Trichosporon</i> spp.	++++					++	+++	++	+++
<i>Malassezia</i> spp.	++++						+	+++	+
<i>Rhodotorula</i> spp.	++++					+	+		+
<b>Moulds</b>									
<i>Aspergillus</i> spp.	+ <sup>b</sup>		++		+	+	++++	++	+++
Zygomycetes			+		+		++++	++	+++
<i>Fusarium</i> spp.	+++			+	++		++	++++	+++
<i>Scedosporium apiospermum</i>	+		++		+		++	+++	++
<i>Scedosporium prolificans</i>			+	+	+		+	+++	+++
<b>Dematiaceous moulds</b>			+++		+		+++	++	++
<b>Dimorphic</b>									
<i>Histoplasma capsulatum</i>	+++	++	+	+	+	+	+++	++	++
<i>Blastomyces dermatitidis</i>			+	+		+	+++	++++	++
<i>Coccidioides immitis</i> complex	++	+	++	+	+	+	++++	+++	+++
<i>Paracoccidioides brasiliensis</i>		+	+				+++	++++	++
<i>Penicillium marneffeii</i>	+++	++	+	++			++++	++	+++
<i>Sporothrix schenckii</i>	+			+			++	++++	+
<b>Other</b>									
<i>Pneumocystis jirovecii</i>		+					++++		+

<sup>a</sup>Predominant sites for recovery are ranked in order of importance and frequency (i.e., ++++ most important or most frequent, + less important or less frequent) based on the most common clinical presentation. Adapted from Pfaller et al.<sup>2</sup>

<sup>b</sup>*Aspergillus terreus* only.

**Table 4-5** Methods and stains available for direct microscopic detection of fungal elements

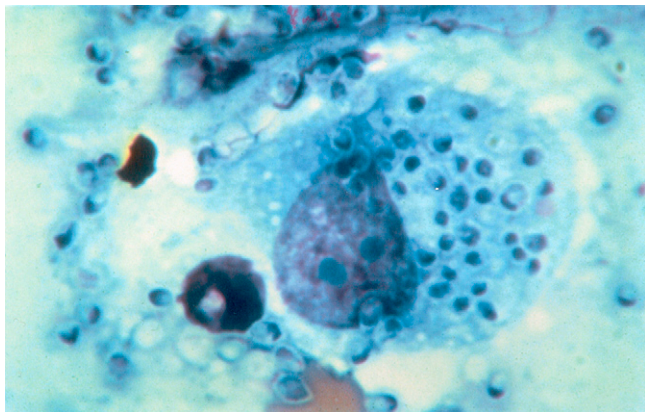
Method/Stain	Use	Comments
Alcian blue stain	Detection of <i>C. neoformans</i> in CSF	Rapid (2 min); insensitive and not commonly used
Calcofluor white stain	Detection of all fungi including <i>P. jiroveci</i>	Rapid (1–2 min); detects fungal cell wall chitin by bright fluorescence. Used in combination with KOH. Requires fluorescent microscope and proper filters. Background fluorescence may make examination of some specimens difficult
Fluorescent monoclonal antibody treatment	Examination of respiratory specimens for <i>P. jiroveci</i>	Sensitive and specific method for detecting the cysts of <i>P. jiroveci</i> . Does not stain the extracystic (trophozoite) forms
Fontana-Masson Stain	Melanin stain for histologic sections	Confirms the presence of melanin in lightly pigmented cells of dematiaceous fungi when present in tissue sections. Useful for distinguishing <i>C. neoformans</i> (positive) from most other yeasts (e.g., <i>Candida</i> spp. are negative for melanin)
Giemsa stain	Examination of bone marrow, peripheral smears, touch preparations, and respiratory specimens	Detects intracellular <i>H. capsulatum</i> and both intracystic and extracystic (trophozoite) forms of <i>P. jiroveci</i> . Does not stain cysts of <i>P. jiroveci</i> . Does stain organisms other than <i>H. capsulatum</i> and <i>P. jiroveci</i>
Gram stain	Detection of bacteria and fungi	Rapid (2–3 min); commonly performed on clinical specimens. Will stain most yeasts and hyphal elements. Most fungi stain Gram positive but some, such as <i>C. neoformans</i> , exhibit stippling or appear Gram negative
Hematoxylin and eosin (H&E) stain	General-purpose histologic stain	Best stain to demonstrate host reaction in infected tissue. Stains most fungi but small numbers of organisms may be difficult to differentiate from background. Useful in demonstrating natural pigment in dematiaceous fungi
India ink	Detection of encapsulated yeasts	Rapid (1 min); insensitive (40%) means of detecting <i>C. neoformans</i> in CSF
KOH treatment	Clearing specimens of cellular debris to make fungi more visible	Rapid (5 min); some specimens may be difficult to clear and require an additional 5–10 min. May produce confusing artifacts. Most useful when combined with Calcofluor white
Methylene blue treatment	Detection of fungi in skin scrapings	Rapid (2 min); may be used in combination with KOH. Largely replaced by Calcofluor white (improved sensitivity and specificity)
Methenamine silver stain (GMS)	Detection of fungi in histologic sections and <i>P. jiroveci</i> cysts in respiratory specimens	Staining of tissue may take up to 1 h. Respiratory specimens more rapid (5–10 min). Best stain for detection of all fungi. Usually performed in cytopathology laboratory
Mucicarmine stain	Histopathologic stain for mucin	Useful for demonstrating capsular material of <i>C. neoformans</i> . May also stain the cell walls of <i>B. dermatitidis</i> and <i>Rhinosporidium sieberi</i>

(Continued)

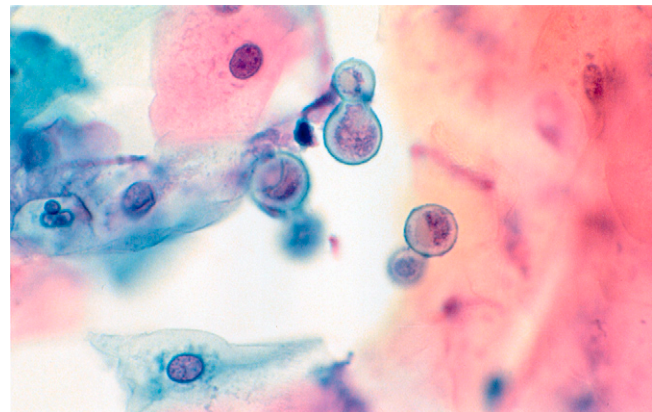
**Table 4-5** Methods and stains available for direct microscopic detection of fungal elements—cont'd

Method/Stain	Use	Comments
Papanicolaou stain (PAP)	Cytologic stain used primarily to detect malignant cells	Stains most fungal elements; yeasts > hyphae. Allows cytologist to detect fungal elements
Periodic acid-Schiff (PAS) stain	Histologic stain for detection of fungi	Stains both yeasts and hyphae in tissue. <i>B. dermatitidis</i> may appear pleomorphic. PAS-positive artifacts may resemble yeast cells
Toluidine blue stain	Examination of respiratory specimens for <i>P. jiroveci</i>	Stains <i>P. jiroveci</i> cysts a purple color. Does stain other fungi. Largely displaced by fluorescent antibody and Calcofluor white treatments
Wright stain	Examination of bone marrow, peripheral smears and touch preparations	Similar to Giemsa stain. Detects intracellular <i>H. capsulatum</i>

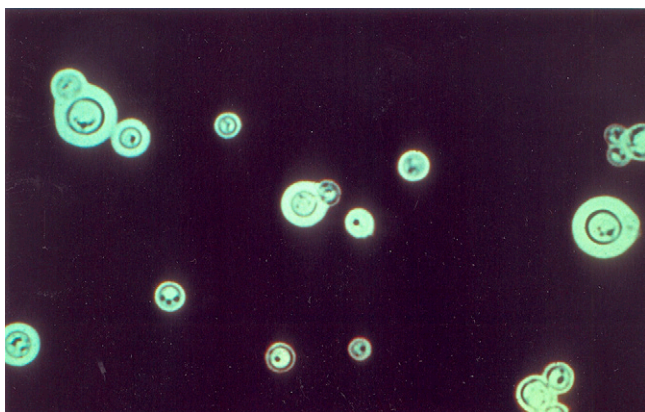
Adapted from Pfaller et al.<sup>2</sup>



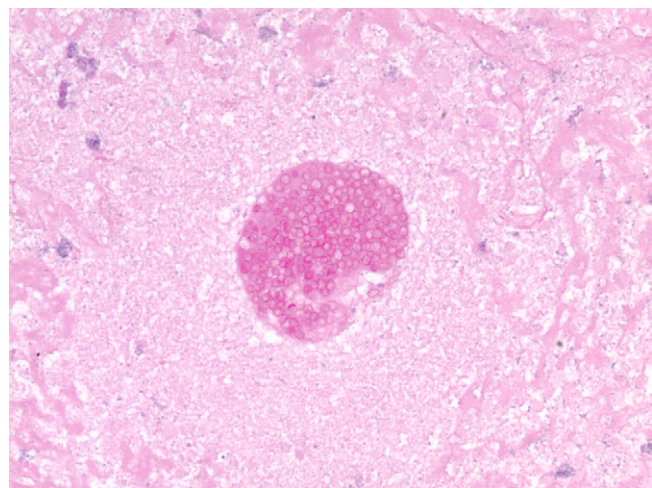
**Figure 4-1** Macrophage containing numerous intracellular yeast forms of *Histoplasma capsulatum* Giemsa stain. Magnification ×1000.



**Figure 4-2** Broad-based budding yeasts of *Blastomyces dermatitidis* in a cytologic preparation. Papanicolaou stain. Magnification ×1000.

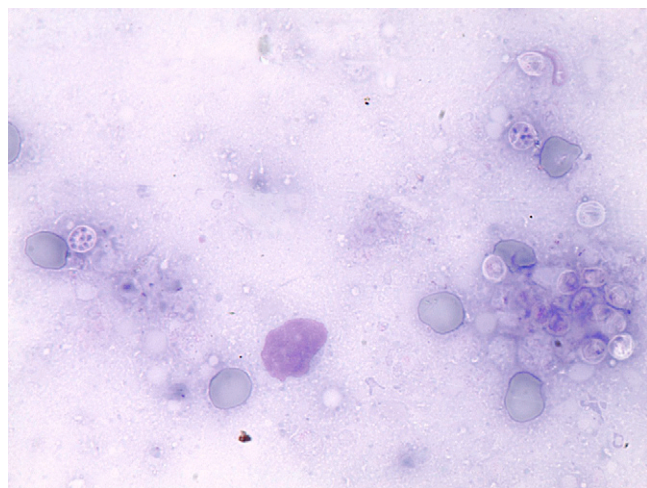


**Figure 4-3** *Cryptococcus neoformans* India ink preparation demonstrating the large capsule surrounding budding yeast cells. Magnification ×1000.

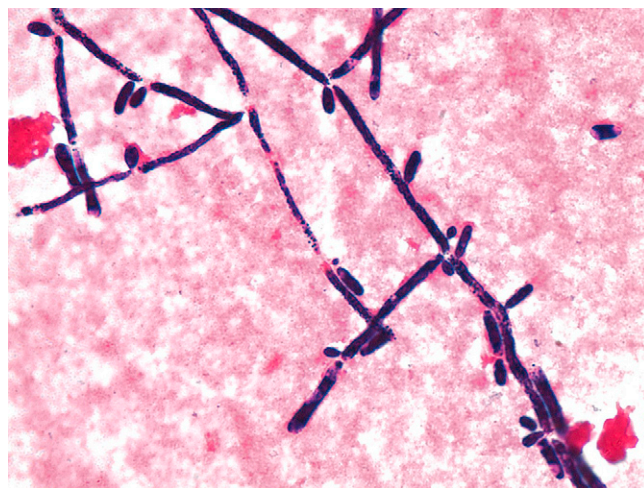


**Figure 4-4** Spherule of *Coccidioides immitis* complex. PAS stain. Magnification ×500.

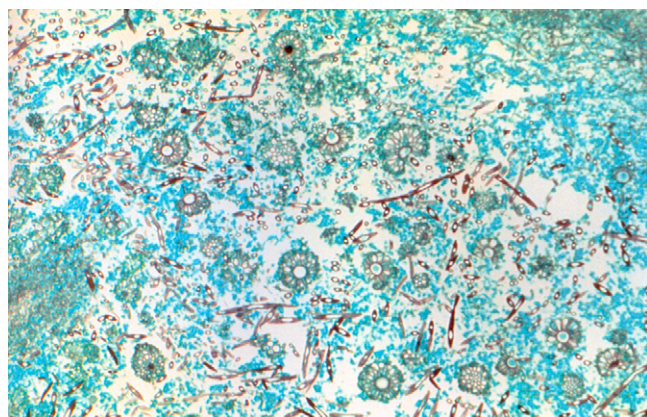




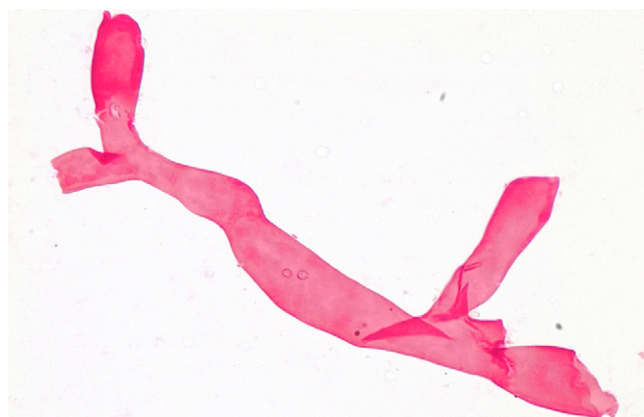
**Figure 4-5** *Pneumocystis jirovecii* in bronchoalveolar lavage fluid. Giemsa stain shows intracystic forms. Magnification  $\times 1000$ .



**Figure 4-7** *Candida tropicalis* blastoconidia and pseudohyphae. Gram stain. Magnification  $\times 1000$ .



**Figure 4-6** *Aspergillus niger* in a cavitary lung lesion showing both hyphae and conidial heads GMS stain. Magnification  $\times 500$ .



**Figure 4-8** Hyphal fragment of *Rhizopus* spp. in pleural fluid demonstrating characteristic broad aseptate hypha that folds back on itself. Gram stain. Magnification  $\times 1000$ .

from blood cultures are clinically significant,<sup>8,9</sup> others such as *Aspergillus* spp. (with the exception of *A. terreus*) and *Penicillium* spp. (with the exception of *P. marneffei*) most probably represent pseudofungemia or contamination.<sup>10-12</sup>

Cultures of any clinical specimens that are positive for any of the endemic dimorphic pathogens (*H. capsulatum*, *B. dermatitidis*, and *C. immitis* complex) are virtually always considered to be clinically significant. Isolation of *Aspergillus* spp. from cultures of respiratory tract specimens is especially problematic, because this organism is common in the environment, and it may colonize the airways of an individual without causing overt disease. The clinical significance of isolation of *Aspergillus* spp. from respiratory tract cultures may be confirmed upon direct microscopic visualization of the organism in viable tissue.

There is now considerable evidence indicating that the interpretation of respiratory tract cultures (e.g., expectorated sputum, BAL) yielding *Aspergillus* spp. may be aided by considering the risk group of the patient<sup>13-15</sup> (Table 4-7). Among patients considered to be at high risk for invasive aspergillosis (IA) (e.g., allogeneic bone marrow transplant (BMT) recipients,

patients with hematologic malignancies, and patients with neutropenia), a positive culture that yields *Aspergillus* spp. is often associated with invasive disease. The positive predictive value (PPV) of a culture positive for *Aspergillus* spp. is lessened for autologous BMT recipients, solid organ transplant recipients, and HIV-infected patients.<sup>13</sup> In addition, the specific identification of the fungus isolated from respiratory culture specimens can also help in determining clinical significance; *Aspergillus niger* is rarely a pathogen, whereas *A. terreus* and *A. flavus* have been shown to be statistically associated with IA when isolated from cultures of respiratory tract specimens.<sup>13</sup>

## Identifying characteristics of fungi

Identification of fungi to genus and species is increasingly important as the spectrum of opportunistic pathogens continues to expand (see Table 4-1). Although the clinical presentation of many fungal infections may be indistinguishable, specific identification of the etiologic agent may have a direct bearing on the management of the infectious process. It is

**Table 4-6** Characteristic features of opportunistic and pathogenic fungi in clinical specimens and in cultures

Fungus	Microscopic morphologic features in clinical specimens	Characteristic morphologic features in culture		Additional tests for identification
		Macroscopic	Microscopic	
<i>Candida</i> spp.	Oval, budding yeasts 2–6 µm in diameter. Pseudohyphae and hyphae may be present	Variable morphology. Colonies usually pasty, white to tan and opaque. May have smooth or wrinkled morphology. Some colonies produce fringes of pseudohyphae at periphery	Clusters of blastoconidia, pseudohyphae and/or terminal chlamydospores in some species	Germ tube production by <i>C. albicans</i> , <i>C. dubliniensis</i> , and <i>C. stellatoidea</i> . Carbohydrate assimilation. Morphology on cornmeal agar. Colony color on CHROMagar. PNA-FISH for <i>C. albicans</i>
<i>Cryptococcus neoformans</i>	Spherical budding yeasts of variable size, 2–15 µm. Capsule may be present. No pseudohyphae or hyphae	Colonies are shiny, mucoid, dome shaped, and cream to tan in color	Budding spherical cells of varying size. Capsule present. No pseudohyphae. Cells may have multiple narrow-based buds	Tests for urease (+), phenoloxidase (+), and nitrate reductase (-). Latex agglutination or EIA test for polysaccharide antigen. Carbohydrate assimilation. Mucicarmine and melanin stains in tissue
<i>Trichosporon</i> spp.	Hyaline arthroconidia, blastoconidia and pseudohyphae 2–4 by 8 µm	Colonies are variably smooth and shiny to membranous, dry and cerebriform	Hyphae, pseudohyphae, blastoconidia and arthroconidia. No chlamydospores	Carbohydrate assimilation and biochemical tests. DNA sequence-based identification increasingly important
<i>Malassezia</i> spp.	Small oval budding yeasts. “Bowling pin” appearance with collarette. Both hyphal and yeast forms may be seen in skin scrapings	Slow-growing colonies. May require fatty acid source (olive oil) for growth	Small oval budding cells with collarette. Rudimentary hyphae	Species may be differentiated by lipid requirement: <i>M. furfur</i> and <i>M. sympodialis</i> , positive; <i>M. pachydermatis</i> , negative. <i>M. furfur</i> will grow in 10% Tween 20 whereas <i>M. sympodialis</i> will not
<i>Aspergillus</i> spp.	Septate, dichotomously branched hyphae of uniform width (3–6 µm). Conidial heads may be seen in cavitory lesions	Varies with species. Colonies of <i>A. fumigatus</i> usually blue-green to gray-green; <i>A. flavus</i> yellow-green; <i>A. niger</i> black; other species vary widely	Varies with species. Conidiophores with enlarged vesicles covered with flask-shaped metulae or phialids. Hyphae are hyaline and septate	Identification based on microscopic and colonial morphology. DNA sequence-based identification increasingly important
Zygomycetes	Broad, thin-walled, pauciseptate hyphae, 6–25 µm with non-parallel sides and random branches. Hyphae stain poorly with GMS stain and often stain well with H&E stain	Colonies are rapid growing, woolly, and gray-brown to gray-black in color	Broad, ribbon-like hyphae with rare septa and irregular sides. Sporangium or sporangiola produced from sporangiophore. <i>Rhizopus</i> spp.: rhizoids at base of sporangiophore	Identification based on microscopic morphologic features

**Table 4-6** Characteristic features of opportunistic and pathogenic fungi in clinical specimens and in cultures—cont'd

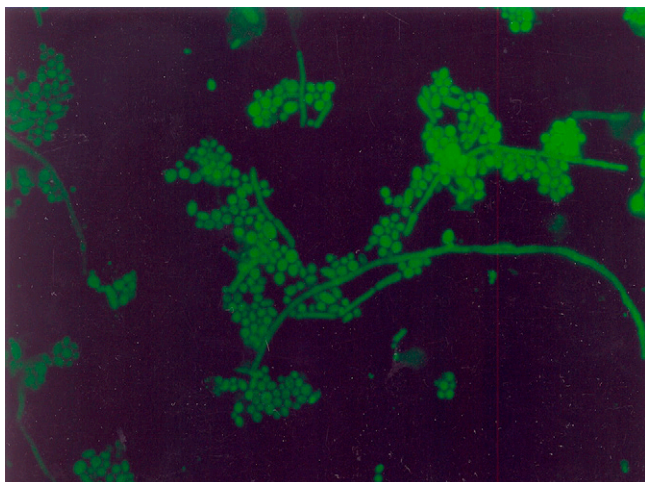
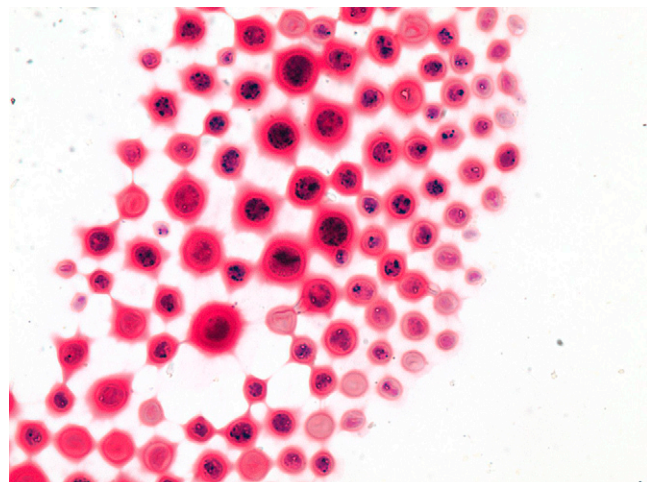
Fungus	Microscopic morphologic features in clinical specimens	Characteristic morphologic features in culture		Additional tests for identification
		Macroscopic	Microscopic	
<i>Fusarium</i> spp.	Hyaline, septate, dichotomously branching hyphae. Angioinvasion is common. May be indistinguishable from <i>Aspergillus</i> spp.	Colonies are purple, lavender or rose-red with rare yellow variants	Both macro- and microconidia may be present. Macroconidia are multicelled and sickle or boat shaped	Identification based on microscopic and colonial morphology DNA sequence-based identification increasingly important
<i>Scedosporium apiospermum</i> (anamorph, asexual stage; <i>Pseudallescheria boydii</i> is the teleomorph or sexual stage)	Hyaline, branching septate hyphae. Angioinvasion is common	Wooly, mouse-gray colonies	Single-celled brownish conidia produced at the tips of annellides ( <i>S. apiospermum</i> ). Cleistothecia containing ascospores may be produced ( <i>P. boydii</i> )	Identification based on microscopic and colonial morphology. May be confused with <i>Aspergillus</i> spp. in tissue
<i>Scedosporium prolificans</i>	Hyaline, branching septate hyphae	Wooly, gray to dark brown. Does not grow on cycloheximide-containing medium	Inflated conidiophores	Based on morphologic appearance. <i>S. prolificans</i> does not have a known sexual stage
Dematiaceous fungi (e.g., <i>Alternaria</i> , <i>Cladosporium</i> , <i>Curvularia</i> )	Pigmented (brown, tan or black) hyphae, 2–6 µm wide. May be branched or unbranched. Often constricted at the point of septation	Colonies are usually rapidly growing, wooly, and gray, olive, black or brown in color	Varies considerably depending on the genus and species. Hyphae are pigmented. Conidia may be single or in chains, smooth or rough and dematiaceous	Identification based on microscopic and colonial morphology
<i>Histoplasma capsulatum</i>	Small (2–4 µm) budding yeasts within macrophages	Colonies are slow growing and white or buff-brown in color (25°C). Yeast-phase colonies (37°C) are smooth, white and pasty	Thin septate hyphae that produce tuberculate macroconidia and smooth-walled microconidia (25°C). Small oval budding yeasts produced at 37°C	Demonstration of temperature-regulated dimorphism by conversion from mould to yeast phase at 37°C. Exoantigen and DNA probe tests
<i>Coccidioides immitis</i> complex	Spherical, thick-walled spherules, 20–200 µm. Mature spherules contain small, 2–5 µm endospores. Arthroconidia and hyphae may form in cavitary lesions	Colonies initially appear moist and glabrous, rapidly becoming downy and gray-white with a tan or brown reverse	Hyaline hyphae with rectangular arthroconidia separated by empty disjunct cells	Exoantigen and nucleic acid probe tests

(Continued)

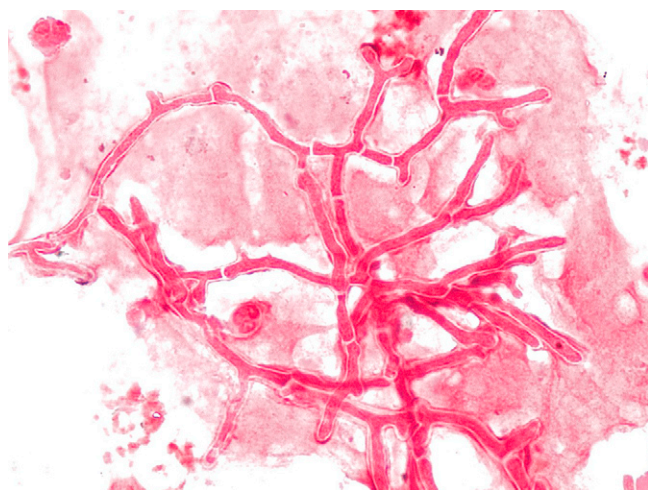


**Table 4-6** Characteristic features of opportunistic and pathogenic fungi in clinical specimens and in cultures—cont'd

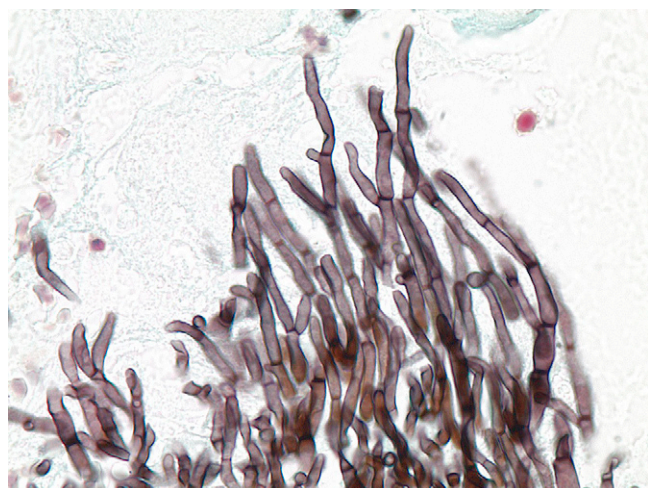
Fungus	Microscopic morphologic features in clinical specimens	Characteristic morphologic features in culture		Additional tests for identification
		Macroscopic	Microscopic	
<i>Blastomyces dermatitidis</i>	Large (8–15 $\mu\text{m}$ ) thick-walled budding yeast cells. The junction between the mother and daughter cells is typically broad-based. Cells may appear multinucleate	Colonies vary from membranous yeast-like colonies to cottony white mould-like colonies at 25°C. When grown at 37°C yeast-phase colonies are wrinkled, folded and glabrous	Hyaline, septate hyphae with one-celled smooth conidia (25°C). Large thick-walled budding yeast at 37°C	Demonstration of temperature-regulated dimorphism; exoantigen and DNA probe tests
<i>Sporothrix schenckii</i>	Yeast-like cells of varying sizes. Some may appear elongated or “cigar shaped.” Tissue reaction forms asteroid bodies	Colonies initially smooth, moist, and yeast-like, becoming velvety as aerial hyphae develop (25°C). Tan to brown pasty colonies at 37°C	Thin branching septate hyphae. Conidia borne in rosette-shaped clusters at the end of the conidiophore (25°C). Variable sized budding yeast at 37°C	Demonstration of thermal dimorphism; exoantigen and DNA probe
<i>Penicillium marneffei</i>	Oval, intracellular yeast cells with septum	Colonies produce diffusible red pigment at 25°C	Septate hyphae with metulae, phialids with chains of conidia in a “paint-brush” distribution (25°C). Yeast cells divide by fission (37°C)	Demonstration of thermal dimorphism
<i>Pneumocystis jiroveci</i>	Cysts are round, collapsed or crescent shaped. Trophozoites seen on special stains	Not applicable	Not applicable	Immunofluorescent stain, GMS, Giemsa, toluidine blue stains

Adapted from Pfaller et al.<sup>2</sup>**Figure 4-9** *Candida tropicalis* blastoconidia and pseudohyphae in CSF stained with Calcofluor white. Magnification  $\times 1000$ .**Figure 4-10** *Cryptococcus neoformans* in CSF. Variable-sized encapsulated budding yeasts seen on Gram stain. Note stippling due to uneven retention of crystal violet stain. Magnification  $\times 1000$ .





**Figure 4-11** *Aspergillus* spp. detected by Gram stain in a tracheal aspirate. Although often Gram positive, this specimen did not retain the crystal violet and appears Gram negative. Magnification  $\times 1000$ .

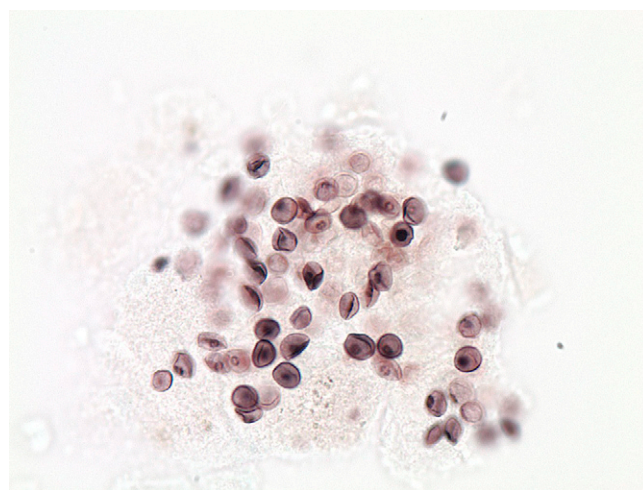


**Figure 4-12** GMS stain showing dichotomously branching septate hyphae characteristic of *Aspergillus* spp. Magnification  $\times 1000$ .

increasingly apparent that one cannot rely on a single therapeutic approach (e.g., administration of amphotericin B) for the management of all, or even most, fungal infections.<sup>16-18</sup> In the case of the more unusual mycoses, specific etiologic identification may provide access to the literature and the experience of others regarding the probable course of infection and response to therapy.

#### Identification of yeasts

Yeasts are usually characterized morphologically as solitary cells that reproduce by simple budding; however, under certain conditions some yeast may form true hyphae, pseudohyphae, capsules, arthroconidia and other reproductive structures. Since *C. albicans* constitutes the vast majority of yeasts recovered from clinical specimens, several rapid and simple tests have been devised to distinguish it from other yeasts.<sup>19,20</sup>



**Figure 4-13** GMS stain of BAL fluid demonstrating cysts of *P. jiroveci*. Magnification  $\times 1000$ .

**Table 4-7** Risk of invasive aspergillosis for patients with respiratory tract cultures positive for *Aspergillus* species, by study and risk characteristics

Risk category	Study, percentage risk (no. of patients with positive culture/total no. of patients)		
	Yu <sup>14</sup>	Horvath <sup>15</sup>	Perfect <sup>13</sup>
High <sup>a</sup>	100 (17/17)	72 (34/47)	57 (117/206)
Intermediate <sup>b</sup>	37 (20/54)	58 (14/24)	15 (228/1510)
Low <sup>c</sup>	0 (0/9)	14 (1/7)	<1 (1/155)

<sup>a</sup>Includes allogeneic BMT recipients, patients with neutropenia, and patients with hematologic cancer.  
<sup>b</sup>Includes autologous BMT and solid organ transplant recipients, patients receiving therapy with corticosteroids, HIV-infected patients, and patients with malnutrition, diabetes, underlying pulmonary disease, or solid organ cancer.  
<sup>c</sup>Includes HIV-infected patients, patients with cystic fibrosis or connective tissue disease, and other non-immunosuppressed patients.

Most recently, a new peptide nucleic acid (PNA) fluorescence in situ hybridization (FISH) test for differentiation of *C. albicans* from non-*albicans* *Candida* species was approved by the FDA for clinical use.<sup>20-24</sup> This PNA-FISH test can be used to identify *C. albicans* directly from blood culture bottles that test positive and in which yeasts are observed by Gram staining. The results are available within 2.5 hours and both single-center and multicenter studies have documented the excellent sensitivity (99–100%) and specificity (100%) of the test.<sup>20,22,24</sup> Importantly, the FISH results are unaffected by the type of blood culture system or broth formulation (e.g., lytic medium and resin- or charcoal-containing medium).<sup>20</sup> It allows physicians to be informed of the yeast's identity along with notification of positive blood culture results. Rapid, accurate identification of *C. albicans* from blood cultures should promote optimal antifungal therapy with the most cost-effective agents (i.e., fluconazole).

Among the more than 100 species of *Candida* that have been identified, five (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*) account for 95–98% of cases of invasive candidiasis (IC).<sup>25</sup> Recent reports indicate that shifts have occurred in the distribution of non-*albicans* species with the emergence of *C. glabrata*, *C. krusei*, *C. lusitanae*, and other less common species.<sup>25,26</sup> Infections with these various species may require different therapeutic considerations.<sup>18,27,28</sup> Due to the pathogenic potential of *C. neoformans*, all encapsulated yeasts from any body site should also be identified. There are several rapid screening tests that may be used for the presumptive identification of *C. neoformans* including the urease test (positive), nitrate test (negative) and production of phenol oxidase (positive).<sup>19</sup> Other important non-*Candida* yeasts are notable due to their broad antifungal resistance profiles.<sup>18,29,30</sup>

### Identification of moulds

In contrast to yeasts, the identification of moulds is largely based upon morphologic features such as gross colony appearance and microscopic morphology (see Table 4-6). Visible growth on agar media may be obtained within 1–5 days for the zygomycetes, most hyaline (light-colored hyphae and conidia) hyphomycetes, and some, but not all, dematiaceous (dark-pigmented hyphae and conidia) fungi.

A variation in colonial morphology that may be either medium or strain dependent precludes the use of this feature as the sole criterion for identification. Surface texture, topography, color, reverse pigmentation, growth at 37°C, and requirements for specific vitamins are all useful characteristics.<sup>31</sup> Definitive identification of most moulds is dependent upon visualization of the microscopic morphology of the fungus. Material must be prepared for microscopic examination in such a way as to minimize any disruption of the relationship of the conidia to their respective reproductive structures. This is usually best accomplished by the use of slide cultures. Determination of cell wall melanin and temperature-regulated dimorphism are also important characteristics. The dimorphic pathogens may also be characterized by immunologic- or nucleic acid probe-based methods in addition to morphology and thermal dimorphism.<sup>32</sup> The typical features of selected filamentous and dimorphic pathogens are listed in Table 4-6.

### Molecular methods for identification of yeasts and moulds

The use of both direct nucleic acid probes and amplification-based molecular approaches provides more rapid and objective identification of yeasts and moulds compared with traditional phenotypic methods.<sup>33-40</sup> These chemiluminescent-labeled DNA probes (AccuProbe, Gen-Probe) are specific for target fungal rRNA and are commercially available for use in clinical laboratories. When applied to a lysate of the organism, the probes have a sensitivity similar to that of the more labor-intensive exoantigen test but demonstrate slightly less specificity, depending on the fungus tested.<sup>37</sup> The probes demonstrate 100% specificity for *C. immitis*;<sup>41</sup> however, specificity is slightly less (99–99.7%) for both *B. dermatitidis* and *H. capsulatum* due to demonstrated cross-reactivity with *Paracoccidioides brasiliensis* and *Chrysosporium* species, respectively.<sup>37,41</sup>

Amplification-based methods are especially useful in identifying non-sporulating moulds that cannot be identified by

conventional methods.<sup>38</sup> Both ribosomal targets and internal transcribed spacer regions have proven useful for the molecular identification of a wide variety of fungi. A major limitation of this approach is the variable quality and accuracy of the existing sequence databases.<sup>35,36</sup> It is anticipated that, with the availability of improved sequencing techniques, broader and more reliable databases, and more readily available kits and software, this technology will be a competitive alternative to the classic mycologic identification methods used for clinically important fungi.<sup>38,42,43</sup>

### Serologic and nucleic acid-based methods of diagnosis

Although culture and histopathology remain the primary means of diagnosing fungal infections, there continues to be a need for more rapid, non-culture methods for diagnosis. Tests for detection of antibodies, rapid detection of specific fungal antigens, metabolic by-products, and fungal species-specific RNA or DNA sequences have the potential to yield rapid diagnostic information that can guide the early and appropriate use of antifungal therapy. Although a great deal of progress has been made in these areas, the true impact on the diagnosis and outcome of invasive fungal infections (IFI) has yet to be realized.<sup>33,44-46</sup>

#### Antibody detection

Serologic tests can provide a rapid means of diagnosing fungal infections, as well as a means to monitor the progression of the infection and the patient's response to therapy by comparing serial determinations of antibody or antigen titers.<sup>38,47-49</sup> Most conventional serologic tests are based on detection of antibodies against specific fungal antigens. Often, this serodiagnostic approach is ineffective because many patients who are at risk for IFI are not capable of mounting a specific antibody response to immunosuppression. In addition, determination of the presence of an acute infection typically requires a comparison of the type and quantity of antibody present in both acute-phase and convalescent-phase serum samples, an exercise that is not helpful during the acute presentation, when therapeutic interventions are being decided.<sup>38,47</sup>

Among the most reliable and widely used conventional serodiagnostic tests in mycology are the antibody tests for histoplasmosis and coccidioidomycosis.<sup>32,50</sup> Both the complement fixation (CF) and the immunodiffusion (ID) tests have been found useful for diagnosis of these infections. Complement fixation titers of >1:32 may be diagnostically significant, whereas lower titers may represent early infection, a cross-reaction, or residual antibodies from a previous infection.<sup>50,51</sup> Immunodiffusion tests are generally less sensitive than CF tests but may be useful in identifying cross-reactions. The ID test for histoplasmosis can give false-positive results if a histoplasmosis skin test has been administered to the patient more than 5–7 days before obtaining sera. Importantly, the CF and ID test detect different antibodies, and both should be performed for maximum diagnostic sensitivity.

Several commercial enzyme-linked immunosorbent assay (ELISA) techniques are now available which detect anti-*Candida* antibodies in an effort to improve the diagnosis of IC.<sup>49,52</sup> These kits have shown sensitivities ranging from 50% to 90% and specificities of ~15–65%.<sup>50</sup> Recently, a new anti-*Candida* antibody (antienolase and intracytoplasmic

antigens) detection ELISA-based kit (Syscan 3, Biomed Ltd, Rockey) was shown to have a sensitivity, specificity, positive predictive value, and negative predictive value (NPV) for IC of 74%, 75%, 62%, and 84% in a group of immunocompetent patients and 15%, 60%, 1.7%, and 93% in an immunocompromised group.<sup>49</sup> Despite a high negative predictive value, it is difficult to see how such testing would be of much value, especially among high-risk patients.

The Platelia *Candida* antibody test (Bio-Rad, Redmond, WA) uses an ELISA format to capture circulating antimannan antibodies in sera from patients, with reported specificity and sensitivity values of 94% and 53%, respectively.<sup>54</sup> When performed simultaneously in combination with a mannan antigen detection test, the method gave a sensitivity of 80% and a specificity of 93%.<sup>52,54</sup> Other authors showed sensitivity and specificity of 59% and 63% respectively for the Platelia antimannan test with an improved sensitivity of 95% and a lower specificity of 53%, when combined with a test for mannanemia.<sup>53</sup>

It appears from these results that the diagnosis of IC cannot be made using a single test for antibodies alone. Rather, a strategy based on detection of mannanemia and antimannan antibodies may prove to be the most useful.<sup>53,55</sup> Furthermore, it appears that regular (at least twice weekly) serum sampling is critical to achieving an early diagnosis of IC.<sup>52-54</sup>

#### Antigen and metabolite detection

Tests to detect fungal antigens or metabolic by-products in serum or other body fluids represent the most direct means of providing a serodiagnosis of IFI.<sup>56-59</sup> Significant advances have been made in recent years; however, for most fungal infections a widely acceptable method is not available. Although several tests for the detection of fungal antigens have been standardized and are now available commercially, issues still remain concerning the sensitivity and specificity of the various

tests in certain patient populations, which populations should be monitored, how often testing should be performed, how the test behaves over time in relation to disease progression or improvement, what testing strategies are the most practical and cost-effective, and what is the true impact of such testing on patient outcome.<sup>59-62</sup>

Presently, the best established and most widely used fungal antigen tests are the latex and enzyme immunoassay (EIA) tests for the detection of the capsular polysaccharide antigen of *C. neoformans*. The commercially available tests for cryptococcal antigen detect >95% of cryptococcal meningitis and approximately 67% of disseminated cryptococcal infections.<sup>56</sup> These antigen tests are well standardized, widely available and supplant India ink (sensitivity <40%) for the diagnosis of cryptococcal meningitis.<sup>56</sup> Another useful antigen test available from a reference laboratory (MiraVista Diagnostics, Indianapolis, IN) is the test for *Histoplasma* antigen. The *Histoplasma* antigen test has been shown to be rapid (<24h), sensitive (55–99%), specific (>98%), and reproducible. The test uses an EIA format and detects a *Histoplasma*-specific polysaccharide antigen present in body fluids. Urine and serum are the most common specimens tested; however, the antigen may be detected in the spinal fluid of 42–67% of patients with *Histoplasma* meningitis and in the alveolar lavage fluid of 70% of patients with AIDS and severe pulmonary histoplasmosis.<sup>63</sup>

Mannan is the major circulating antigen in patients with IC. Detection of mannan is complicated by rapid clearance from the patient's sera and binding by antimannan antibody. Although circulating mannan may be detected by several methods (Table 4-8), a dissociation of antigen–antibody complexes is required for optimal sensitivity.<sup>7</sup> Sensitivities of 25–100% and specificities of 92–100% have been reported with EIA assays for mannan detection<sup>7,52,54,55,58,64,65</sup> (see Table 4-8). An early commercial system to detect mannan used a latex

**Table 4-8** Detection of antigenemia in patients with invasive candidiasis

Antigen Detected	Method (Manufacturer)	No. Patients	%		Reference
			Sens	Spec	
Enolase	Sandwich EIA	170	75	96	68
Mannan	ELISA	209	74	100	58
Mannan	LA (Pastorex)	72	25	100	66
Mannan	EIA (Platelia)	193	40	98	52
Mannan (α)	EIA (Platelia)	144	69	98	65
Mannan (β)	EIA	144	69	95	65
Mannan (α and β)	EIA	144	85	95	65
Mannan	EIA (Platelia)	60	86	79	53
Mannan	EIA	34	60	92	67
Mannan	LA (Pastorex)	79	26	100	69

Abbreviations: EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; LA, latex agglutination; Sens, sensitivity; Spec, specificity.



agglutination (LA) format (Pastorex *Candida* test, Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France) and demonstrated poor sensitivity (0–25%) due to the rapid clearance of mannan from patients' sera and the insensitive LA format.<sup>66</sup> More recently, the Platelia *Candida* antigen test (BioRad) uses a monoclonal antibody-based double sandwich EIA format with a resultant increase in sensitivity and a limit of detection of 0.1 ng of mannan per ml of serum.<sup>52,52,55</sup> Clinical evaluations of the Platelia *Candida* antigen test report sensitivities ranging from 40% to 86% and specificities from 79% to 98%<sup>52,54,58,64,67</sup> (see Table 4-8). Virtually every study evaluating the detection of the mannanemia has shown that multiple serial samples are required to overcome the rapid clearance of mannan from patients' sera and to optimize diagnostic sensitivity.

Sendid et al<sup>65</sup> took advantage of differences in clearance of  $\alpha$ -mannan (rapid) and  $\beta$ -mannan (slower) to demonstrate that simultaneous detection of both forms of mannan improves the sensitivity of a test for mannanemia from 69%, when either  $\alpha$ -mannan or  $\beta$ -mannan was tested for alone, to 85% when both forms were tested for in the diagnosis of IC. The specificity of the single tests was 98% ( $\alpha$ -mannan) and 95% ( $\beta$ -mannan) and for the combined test was 95%. They also demonstrated that mannanemia preceded early clinical symptoms and isolation of *Candida* in culture by an average of 4.7 days. Although the current Platelia *Candida* antigen test is specific for  $\alpha$ -mannan only, these results suggest that joint detection of both epitopes is a rational approach that contributes to increases in the sensitivity and timeliness of diagnosis.

Although a test for  $\beta$ -mannan is not yet available, Sendid and colleagues<sup>52,54,55</sup> have applied a similar rationale to the use of the Platelia *Candida* antibody test in combination with the Platelia *Candida* antigen test to maximize the ability to diagnose IC. As discussed previously, simultaneous testing for mannanemia and antimannan antibodies resulted in an improved sensitivity from 40–53% with the single tests to 80% with combined testing.<sup>54</sup> Specificity remained high (93%) with the combined testing strategy. Whereas the Platelia *Candida* antigen test is specific for  $\alpha$ -mannan, the Platelia antibody test detects antibodies against the whole mannan oligomannose repertoire containing both  $\alpha$ - and  $\beta$ -mannan epitopes.<sup>54,65</sup> These and other investigators emphasize the importance of regular (twice weekly) serial monitoring of at-risk patients in maximizing the sensitivity of serodiagnostic testing for IC.<sup>53,54</sup>

Among the various protein antigen targets, perhaps the most promising for diagnosis of IC was enolase. This 48 kDa antigen was known to circulate in the absence of fungemia and correlated with deep tissue infection. A commercial assay was developed (Directigen, Becton-Dickenson) using an antienolase monoclonal antibody and a double sandwich antigen capture format. In a prospective clinical trial conducted in four medical oncology centers, Walsh et al<sup>68</sup> concluded that enolase antigenemia was a marker for deep tissue invasion by *Candida* spp. even in the absence of fungemia. Furthermore, the serum enolase immunoassay complemented, rather than replaced, blood cultures for the diagnosis of IC. The assay was very specific (96%) and when multiple samples were tested per patient the sensitivity was 85% for patients with proven deep tissue infection and 64% in proven cases of candidemia. Both Gutierrez et al,<sup>66</sup> using the Directigen test, and Mitsutake et al,<sup>69</sup> using a dot-immunobinding assay,

confirmed the value of enolase detection in the diagnosis of IC. Unfortunately, the Directigen test for enolase is no longer available and there are no other commercial sources for enolase detection methods.

*Candida* produces large amounts of D-arabinitol in culture and during the course of IC.<sup>7,70</sup> It is notable that *C. glabrata* and *C. krusei* do not produce this metabolite. Several groups have shown that serum D-arabinitol concentrations and serum D-arabinitol/creatinine ratios are higher in individuals with IC than in uninfected or colonized controls.<sup>71</sup> Elevated D-arabinitol/creatinine ratios in serum or urine from patients with IC were detected, often prior to positive blood cultures, and the ratios have been correlated with therapeutic response.<sup>7</sup> Walsh et al<sup>71</sup> used an automated enzymatic method of detecting D-arabinitol in serum in a large prospective multicenter study of high-risk neutropenic cancer patients and BMT recipients and demonstrated that most patients (74%) with IC had high serum D-arabinitol/creatinine ratios and that change in the ratios over time correlated with responses to antifungal therapy. The results of a large population-based study<sup>70</sup> of unselected patients with candidemia showed that serum D-arabinitol/creatinine measurements were as sensitive, specific, and timely for the initial detection of candidemia in patients with a broad range of underlying conditions as had previously been reported in cancer patients and other special populations.<sup>70,71</sup>

$\beta$ -1-3-Glucan (BG) is an important component of the cell wall of *Candida*, *Aspergillus*, and many other pathogenic fungi. Although BG is not immunogenic, the fact that it can be found circulating in the bloodstream of patients with IFI has been exploited for use diagnostically and as a surrogate marker of infection.<sup>72</sup> The Fungitell BG assay (Associates of Cape Cod Inc., Falmouth, MA), is an FDA-approved commercially available colorimetric assay that can indirectly determine the concentration of BG in the serum. The detection system is based on the activation of a BG-sensitive proteolytic coagulation cascade, the components of which are purified from the horseshoe crab. The assay can measure picogram amounts of BG and has been used to demonstrate the presence of the polysaccharide in the serum of patients with IC and IA, but not cryptococcosis or zygomycosis (organisms lack BG)<sup>72-74</sup> (Table 4-9). Several studies have demonstrated a high degree of sensitivity and specificity in the diagnosis of IC (78–97% sensitivity and 88–100% specificity) and IA (50–87.5% sensitivity and 81–89% specificity).<sup>67,69,72,75-78</sup> BG is also detectable in patients with infections caused by species of *Fusarium*, *Trichosporon*, *Saccharomyces*, and *Acremonium*.<sup>79</sup>

The Fungitell BG assay has been evaluated for the early diagnosis of IFI in patients with hematologic malignancies<sup>72,76</sup> and in a multicenter study of patients with IFIs and healthy control subjects.<sup>78</sup> In the latter study sera were obtained from 170 fungal infection-negative control subjects and from 163 patients with proven or probable IFI diagnosed at one of six participating medical centers. Overall, the sensitivity and specificity of the assay were 69.9% and 87.1% respectively, with a PPV of 83.8% and a NPV of 75.1%. The sensitivity of the BG test was 81.3% among the 107 patients with proven IC and 80% in the 10 patients with IA. Another study of patients with acute myelogenous leukemia suggested that the sensitivity, specificity, and PPV of the assay increased significantly if sera were obtained twice weekly.<sup>76</sup> Obtaining multiple samples increased the sensitivity, PPV, and NPV of the BG assay to



**Table 4-9**  $\beta$ -D-Glucan in the diagnosis of proven or probable invasive fungal infections<sup>a</sup>

Reference	No. patients	%	
		Sens	Spec
69	79	84	88
67	34	78	92
76	283	100	90
77	76	93	77
78	333	70	87
72	40	88	90

<sup>a</sup>Infections with various pathogens including *Candida*, *Fusarium*, *Trichosporon*, and *Aspergillus* spp.  
Abbreviations: Sens, sensitivity; Spec, specificity.

>98% for subjects with leukemia who were receiving antifungal prophylaxis.

Pickering et al<sup>77</sup> tested sera from healthy blood donors and patients with candidemia and found a sensitivity and specificity of 92.9% and 100%, respectively. When bacteremic patients were included in their assessment of the performance of the BG assay, the specificity and PPV fell to 77.2% and 51.9% respectively, due to a high number of false-positive results, especially in samples from patients with Gram-positive bacteremia. They found that hemolysis would cause false-positive BG test results and that high concentrations of bilirubin and triglycerides were inhibitory and would cause false-negative results. Other causes of false-positive BG test results included hemodialysis with cellulose membranes, patients treated with intravenous immunoglobulins, albumin, coagulation factors or plasma protein factor, or patients exposed to gauze or other materials that contain glucans.<sup>77</sup> Thus a negative BG test result may be useful for ruling out an IFI due to most fungal pathogens with the exception of cryptococci or the zygomycetes; however, a single positive result should be confirmed by testing another specimen (two consecutive positive tests) and a thorough review for potential sources of false positivity should be conducted.

In a study designed to assess the benefit of monitoring patients for the presence of both BG and galactomannan (GM), Pazos et al<sup>72</sup> found that the combination of the two tests improved both specificity (to 100%) and PPV (to 100%) for the diagnosis of IA, without affecting sensitivity or NPV. Although both tests were useful for early diagnosis of IA, the BG test was positive earlier than the GM assay. Testing should be performed with a minimal amount of sample manipulation and sequential positive results should be required for a “true-positive” test result.<sup>76</sup> A positive BG result should prompt further diagnostic work-up that may include testing for GM or the use of the polymerase chain reaction (PCR) to define and identify the IFI.

Galactomannan is an important component of the cell wall of *Aspergillus* spp. Similar to mannan in IC, GM has been detected in biologic fluids (serum, urine, BAL fluid) obtained

from patients with IA.<sup>80</sup> Detection of GM for diagnosis of IA has been facilitated by the development of monoclonal antibody-based EIA methods which can detect as little as 0.5–1 ng of GM per ml of serum.<sup>47,80</sup> In 2003, the Platelia *Aspergillus* EIA test (Bio-Rad) was approved by the US FDA for use in the diagnosis of IA in BMT recipients and in patients with leukemia.

GM EIA results are reported as a ratio between the optical density (OD) of the patient's serum sample and that of a control with a low, but detectable, amount of GM and data expressed as the serum GM index (GMI). Most published studies use a cut-off GMI of <1.0 as a negative value, a value greater than 1.5 as positive and those between 1.0 and 1.5 as indeterminate.<sup>81</sup> The data submitted to the FDA used a cut-off of 0.5 as positive and required that the test be positive on two aliquots of the same sample, rather than multiple samples testing positive.<sup>82</sup> These criteria resulted in a sensitivity of 80.7% and specificity of 89.2% for the diagnosis of IA in a multicenter study conducted on serially collected serum samples from 179 BMT recipients and patients with leukemia (31 with IA).<sup>82</sup>

In contrast to the data submitted to the FDA, several studies have used GMI values of 1.0 as a positive cut-off and have required that two consecutive samples must test positive to declare a positive assay<sup>81</sup> (Table 4-10). It is now clear that the reported results with this test can be influenced by the extent of invasive aspergillosis at the time of diagnosis, the prevalence of aspergillosis among the patients studied, exposure of the patient to mould-active antifungals, the cut-off ratio used, and whether multiple consecutive positive tests were or were not required for significance.<sup>47,80,81</sup>

In a meta-analysis of 27 studies in which GM EIA was used to diagnose IA, Pfeiffer et al<sup>81</sup> found a pooled sensitivity and specificity of 71% and 89%, respectively, for the diagnosis of proven IA (see Table 4-10). The high NPV (98%) and low PPV (26%) suggest that the GM assay is good for ruling out disease but is less useful for confirming the diagnosis of IA.<sup>61</sup> The test was found to be most useful in patients with hematologic malignancy or who have undergone BMT than in solid organ transplant recipients<sup>81</sup> (see Table 4-10). Irrespective of the GMI threshold employed, it is apparent that in approximately two-thirds of patients with IA, circulating GM can be detected at a mean of 8 days before diagnosis by another means.<sup>80</sup> When performed in serial fashion, a gradual increase in GMI in consecutive samples is a very strong indication of infection and should be considered when interpreting the results. Likewise, the course of the antigen seems to correlate well with outcome and could be important in monitoring therapeutic response.<sup>83</sup> False-positive GM assay results have been reported in children,<sup>84</sup> in patients receiving piperacillin-tazobactam<sup>85-87</sup> and amoxicillin-clavulanate,<sup>88</sup> in BAL fluid containing Plasmalyte<sup>88</sup> and possibly in patients consuming GM-rich foods.<sup>80</sup>

Studies of combining GM with other diagnostic modalities such as computed tomography (CT) scans of the chest suggest that this approach may be useful in establishing a likely diagnosis of IA.<sup>45,48,57,89-91</sup> Busca et al<sup>57</sup> demonstrated that sequential (twice-weekly) GM detection combined with early radiologic evaluation (chest CT) were useful tools to detect minimal changes of IA and initiation of antifungal therapy. Maertens et al<sup>48</sup> assessed the feasibility of daily GM monitoring and clinical evaluation coupled with high-resolution thoracic CT and bronchoscopy with lavage in identifying patients who

**Table 4-10** Sensitivity and specificity of the galactomannan (GM) assay for diagnosis of proven invasive aspergillosis<sup>a</sup>

Patient group	No. patients	%	
		Sens	Spec
All studies <sup>b</sup>	4284	71	89
Hematologic malignancy	2960	70	92
Bone marrow transplant recipients	903	82	86
Solid organ transplant recipients	224	22	84
Positive GM cut-off value			
0.5	352	27	79
1.0	1705	79	87
1.5	2227	68	92

<sup>a</sup>Data compiled from Pfeiffer et al.<sup>81</sup><sup>b</sup>A total of 27 studies.

should receive preemptive antimould antifungal therapy. No undetected cases of IA were identified, although one case of zygomycosis was missed. Pazos et al<sup>72</sup> have shown that using GM in combination with BG testing provided an increase in specificity for the diagnosis of IA compared to either test alone. Musher et al<sup>89</sup> found that GM EIA and quantitative PCR added to the sensitivity of BAL for diagnosing IA in high-risk patients

### Nucleic acid detection

At present, most of the research has been focused on the diagnosis of IC<sup>6,7,47,64</sup> and IA;<sup>92-94</sup> however, PCR has also been applied to the diagnosis of other IFI.<sup>95</sup> It should be noted, however, that despite a great deal of interest in molecular approaches to the diagnosis of infectious diseases, only 20% of US clinical microbiology laboratories perform *any* molecular tests for infectious diseases and the bulk of those laboratories offer only molecular testing for sexually transmitted diseases.<sup>96,97</sup> Furthermore, molecular methods are used in only 5% of laboratories providing diagnostic services in medical mycology.<sup>97</sup>

PCR-based methods for the diagnosis of IFI have been applied to a variety of specimen types to include whole blood, serum, tissue, BAL fluid, and CSF. Target sequences vary widely but include genus- and species-specific variable regions as well as highly conserved regions of the fungal genome.<sup>6,7</sup> Both single (e.g., hsp90, lanosterol demethylase, chitin synthase, actin) and multicopy (e.g., ribosomal, intergenic transcribed spacer regions (ITS), mitochondrial) gene targets have been studied, although molecular diagnostic methods targeting multicopy genes generally have better sensitivity than those targeting single copy genes<sup>7</sup> (Table 4-11). The use of multicopy ribosomal (18S rRNA, 28S rRNA, 5.8S rRNA and ITS) targets offers the

potential for sensitive panfungal markers for detection of IFI, followed by identification at the genus or species level.<sup>6,7</sup>

PCR amplicon detection methods vary widely but most often employ capture probes in an ELISA format. More recently, real-time PCR platforms have employed rapid target amplification coupled with immediate fluorescent detection of the amplicon and melting curve analysis to provide both detection and identification of the fungal pathogen.<sup>47,98,99</sup> Real-time PCR is both rapid and quantitative and has the additional advantages of using a single-tube closed system to limit contamination, a non-gel based target detection method, and improved standardization via the availability of generic kits and analyte-specific reagents (ASRs).<sup>100</sup> Irrespective of the technology used, most reports in the literature indicate that the sensitivity of PCR-based diagnosis is equal to or better than other currently used diagnostic techniques.<sup>6,7,47</sup>

PCR-amplified *Candida*-specific DNA has been recovered from blood and other body fluids obtained from infected patients.<sup>6,7</sup> *Candida*-specific targets include the lanosterol demethylase gene (LIA1), the actin gene, a chitin synthase gene, hsp90, and a secreted aspartyle proteinase (SAP) gene (see Table 4-11). In addition to *Candida*-specific targets, numerous investigators have targeted the multicopy broad-range panfungal genes such as the 18S, 5.8S, and 28S ribosomal DNA genes (rDNA), and the ITS regions within the rDNA gene cluster<sup>6,7</sup> (see Table 4-11). Using these approaches, detection of as few as 2–10 cells per ml of blood has been reported, although most assays do not approach this level of sensitivity in clinical samples.<sup>7</sup> The true sensitivity of PCR-based methods for the diagnosis of IC is unknown, but sensitivities of 48–100% have been reported (see Table 4-11). Importantly, PCR-based tests for *Candida* (or panfungal) DNA in blood are negative in most subjects with gastrointestinal colonization with *Candida* species and the specificity of these tests is quite high<sup>61,101</sup> (see Table 4-11).

PCR has also been used successfully for early detection of *Aspergillus* DNA in peripheral blood and in BAL fluid.<sup>92-94,102</sup> Sensitivities range from 64% to 100% in patients with proven/probable IA (Table 4-12). Specificities also vary from 65% to 98–100% (see Table 4-12). False-positive results may be seen when BAL fluid is tested, most likely due to the transient presence of conidia in the respiratory tract. The use of whole blood, serum or plasma may be preferable to the use of respiratory tract specimens, because contamination with conidia is much less likely.<sup>102</sup> The combination of GM antigen testing and either PCR or nucleic acid sequence-based amplification (NASBA) using serum or BAL fluid has proven to be useful in regular screening for IA in patients with hematologic disorders.<sup>45,89,92,93,104</sup> The combined use of PCR and GM assays increased the sensitivity and negative predictive values of each individual test to 83.3% and 97.6%, respectively, for the early diagnosis of IA in patients with hematologic malignancies.<sup>92,93</sup> Likewise, the combination of GM and NASBA testing improved the sensitivity of diagnosis to 100%.<sup>104</sup> Despite promising reports, PCR for the diagnosis of IFI has not been widely used in clinical settings.<sup>96,97,103,105</sup> Aside from infection due to *Candida*, the relatively low numbers of patients suspected of having IFI effectively precludes the establishment of a laboratory devoted solely to the molecular diagnosis of IFI.<sup>105</sup> Furthermore, it has not been demonstrated convincingly that PCR can compensate for the limitations of culture and histopathology in the rapid diagnosis of IFI and produce a definitive impact on IFI-related mortality.<sup>103,105</sup>

**Table 4-11** PCR for *Candida* spp. using genus/species-specific and panfungal targets<sup>a</sup>

Target gene	Detection method	Sample	No. patients	Sens (%)	Spec (%)
<b>Genus/species-specific</b>					
Actin	Probe	Serum	43	79	100
Chitin synthase	Probe	Blood	50	93	100
LIA1	Southern	Blood	80	71–100	95–97
LIA1	PCR-REA	Blood	31	98	NA
SAP	PCR-EIA	Blood	124	100	100
<b>Panfungal</b>					
18SrDNA	Probe	Blood	121	88–100	97
18SrDNA	Southern	Blood	200	48	100
18SrDNA	Probe	Blood	105	95	97
18SrDNA	Probe	Blood	97	100	97
18SrDNA	Probe	Blood	59	100	100
ITS	Sequencing	Blood	225	72	91
ITS	Sequencing	Blood	42	88	100

<sup>a</sup>Data compiled from Chen et al,<sup>6</sup> and Yeo and Wong.<sup>7</sup>**Table 4-12** PCR for diagnosis of invasive aspergillosis

Gene target	Detection method	Sample	No. patients	% Sens Spec	
				Sens	Spec
18SrDNA	Gel	Blood	140	100	89
18SrDNA	Probe	Blood	84	100	65
18SrDNA	Probe	Blood	92	100	73
18SrDNA	Nested-PCR	BAL	67	100	93
18SrDNA	Nested-PCR	Blood	218	92	81
18SrDNA	Probe	Blood	122	79	92
18SrDNA	Nested-PCR	Blood	165	64	64
18SrDNA	Molecular beacon	BAL	99	67	100
Mitochondrial	PCR-ELISA	Serum	201	64	90
18SrDNA	Probe	Plasma	96	64	87
18SrDNA	PCR-ELISA	Blood	121	75	96
28SrDNA	Probe	Blood	203	92	95

Data compiled from refs 6, 7, 75, 89, 92–95, 102, 104.

**Table 4-13** Antifungal susceptibility testing: interpretive breakpoints using CLSI methods<sup>a,b</sup>

Antifungal agent	Interpretive breakpoints (µg/ml)			Comments
	S	SDD/I	R	
Fluconazole	≤8	16–32	≥64	Follows 90–60 Rule of clinical response (105); <sup>c</sup> 90.6% of 13,338 isolates ≤8 µg/ml (111)
Itraconazole	≤0.12	0.25–0.5	≥1	Follows 90–60 Rule of clinical response; 96% of 7299 isolates ≤1 µg/ml (113)
Flucytosine	≤4	8–16 <sup>d</sup>	≥32	Follows 90–60 Rule of clinical response; 95% of 803 isolates ≤4 µg/ml (113)
Voriconazole	≤1	2	≥4	Follows 90–60 Rule of clinical response; 98% of 13,338 isolates ≤1 µg/ml (112)
Anidulafungin	≤2	NA	NA	99% of 2235 isolates ≤2 µg/ml (113)
Caspofungin	≤2	NA	NA	99% of 2656 isolates ≤2 µg/ml (113)
Micafungin	≤2	NA	NA	100% of 2656 isolates ≤2 µg/ml (113)
Posaconazole	NA	NA	NA	98% of 2171 isolates ≤1 µg/ml (114)
Amphotericin B	≤1		>1	Use Etest

<sup>a</sup>Pertains to *Candida* spp. only.  
<sup>b</sup>Abbreviations: S, susceptible; SDD, susceptible dose dependent; I, intermediate; R, resistant; NA, not available.  
<sup>c</sup>90–60 Rule: infections due to susceptible isolates respond to appropriate therapy 90% of the time whereas infections due to resistant isolates (or infections treated with inappropriate antimicrobials) respond 60% of the time.  
<sup>d</sup>MICs of 8–16 µg/ml for flucytosine are considered intermediate not SDD.

## Antifungal susceptibility testing

The Clinical and Laboratory Standards Institute (CLSI) Subcommittee for Antifungal Testing has developed standardized broth microdilution (BMD)<sup>106,107</sup> and disk diffusion methods<sup>108</sup> for in vitro susceptibility testing of yeasts and moulds. These methods are reproducible and accurate, and provide clinically useful information that is comparable to that of antibacterial testing.<sup>109,110</sup>

Interpretive breakpoints for seven systemically active antifungal agents (fluconazole, itraconazole, voriconazole, flucytosine (5FC), anidulafungin, caspofungin, and micafungin) have been developed by considering data relating the minimum inhibitory concentrations (MICs) to known resistance mechanisms, MIC distribution profiles, pharmacokinetic (PK) and pharmacodynamic (PD) parameters, and the relationship between in vitro activity (MIC or zone diameter) and clinical outcome<sup>109,111,112</sup> (Table 4-13). Although interpretive breakpoints have not been established for posaconazole, the CLSI Subcommittee has come to a consensus on standardized methods for this agent, and it is expected that interpretive breakpoints will be established in the near future.<sup>113,114</sup>

Establishing a clinical correlation between in vitro susceptibility tests and clinical outcome has been difficult (Table 4-14). Antifungal susceptibility testing can be said to predict the outcome of treatment consistent with the “90–60 Rule.”<sup>109,111,112</sup> According to this rule, infections due to susceptible isolates respond to therapy ~90% of the time, whereas infections due to resistant isolates respond to therapy ~60% of the time (see Table 4-14). Thus, low MICs are not entirely

predictive of clinical success, and high MICs help to predict which patients are less likely to have a favorable response to a given antifungal agent. The 90–60 Rule reflects the fact that the in vitro susceptibility of an infecting organism to the antifungal agent is only one of several factors that may influence the likely success of therapy for an infection.<sup>103,109</sup> Despite considerable progress, it remains to be seen how useful antifungal susceptibility testing will be in guiding therapeutic decision making. Guidelines for the use of laboratory studies, including antifungal susceptibility testing, have been developed<sup>109</sup> (Table 4-15). Future efforts will be directed toward further validation of interpretive breakpoints for established antifungal agents and developing them for newly introduced systemically active agents.

## Conclusion

The infectious fungi now constitute one of the most important threats to the survival of immunocompromised hosts. There is little doubt that in addition to *C. albicans* and *A. fumigatus*, a vast array of fungi, previously considered to be non-pathogenic, may serve as significant human pathogens. Recognition of these emerging fungal pathogens has resulted in a better understanding of their clinical presentation and response to the available therapeutic measures. Conventional laboratory-based methods for diagnosis of fungal infection remain useful but are often slow and lack sensitivity. Clearly, there is a need for improved diagnosis and management of these difficult infections.



**Table 4-14** Correlations of susceptibility testing with outcome for candidal and bacterial infections<sup>a,b,c</sup>

Organism group	No. studies	No. patients	Cases with successful outcome % (no. cases/total) by susceptibility class <sup>d</sup>		
			S	R	P value
<i>Candida</i> <sup>e</sup>	12	1295	85 (841/993)	42 (72/172)	<0.001
Bacteria <sup>f</sup>	12	5447	89 (4521/5081)	59 (215/366)	<0.001

<sup>a</sup>Adapted from Rex and Pfaller<sup>109</sup> and Pfaller et al.<sup>111</sup>  
<sup>b</sup>Antifungal testing performed according to CLSI M27-A2.  
<sup>c</sup>Susceptibility to antibacterial agents determined by MIC, zone diameter, AUC/MIC ratio or peak/MIC ratio.  
<sup>d</sup>Outcome measurement varied from clinical and/or microbiologic response to therapy.  
<sup>e</sup>Includes mucosal, fungemia, meningitis, and disseminated infections treated with fluconazole.  
<sup>f</sup>Includes bacteremia, otitis, and severe infections treated with various agents including cephalosporins,  $\beta$ -lactamase inhibitor combinations, aminoglycosides, and fluoroquinolones.

**Table 4-15** Recommendations for studies of fungal isolates in the clinical laboratory<sup>a</sup>

Clinical setting	Recommendation
Routine	Species-level identification of all <i>Candida</i> isolates from deep sites Genus-level identification of moulds (species level preferred for <i>Aspergillus</i> ) Routine antifungal testing of fluconazole and flucytosine against <i>Candida</i> isolated from blood and normally sterile body fluids and tissue
Oropharyngeal candidiasis	Determination of susceptibility to fluconazole and itraconazole may be helpful but not routinely necessary Susceptibility testing may be useful for patients unresponsive to azole therapy
Invasive disease with clinical failure of initial therapy	Consider susceptibility testing as an adjunct: - <i>Candida</i> species and amphotericin B, fluconazole, voriconazole or echinocandins - <i>C. neoformans</i> and fluconazole, flucytosine, or amphotericin B - <i>H. capsulatum</i> and fluconazole Consultation with an experienced microbiologist recommended
Infection with species with high rates of intrinsic or acquired resistance	Susceptibility testing not necessary when intrinsic resistance is known (e.g., <i>C. krusei</i> vs fluconazole; <i>A. terreus</i> vs amphotericin B) Select therapy based on literature When high rates of acquired resistance (e.g., <i>C. glabrata</i> and fluconazole) monitor closely for signs of failure and perform susceptibility testing
New treatment options (e.g., caspofungin, voriconazole) or unusual organisms	Role of susceptibility testing to be determined Select therapy based on published consensus guidelines and review of survey data on the organism–drug combination in question
Patients who respond to therapy despite being infected with an isolate later found to be resistant	Best approach not clear Take into account severity of infection, patient immune status, consequences of recurrence of infection, etc. Consider alternative therapy for infections with isolates that appear to be highly resistant to therapy selected
Mould infections	Susceptibility testing not recommended as a routine Interpretive criteria have not been established

(Continued)

**Table 4-15** Recommendations for studies of fungal isolates in the clinical laboratory—cont'd

Clinical setting	Recommendation
Selection of susceptibility testing method	<p>Standardized methods</p> <p>CLSI broth-based methods:</p> <ul style="list-style-type: none"> <li>- Yeasts; M27-A2</li> <li>- Moulds; M38-A</li> </ul> <p>Commercial BMD and automated methods:</p> <ul style="list-style-type: none"> <li>- YeastOne Colorimetric</li> <li>- VITEK 2 Yeast Susceptibility Test</li> </ul> <p>Agar-based methods:</p> <ul style="list-style-type: none"> <li>- Etest, numerous agents, yeasts and moulds</li> <li>- Disk (fluconazole, voriconazole), CLSI M44-A method for yeasts</li> </ul>

<sup>a</sup>Adapted from Rex and Pfaller.<sup>109</sup>

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# Histopathology of fungal infections

Vicki J. Schnadig, Gail L. Woods

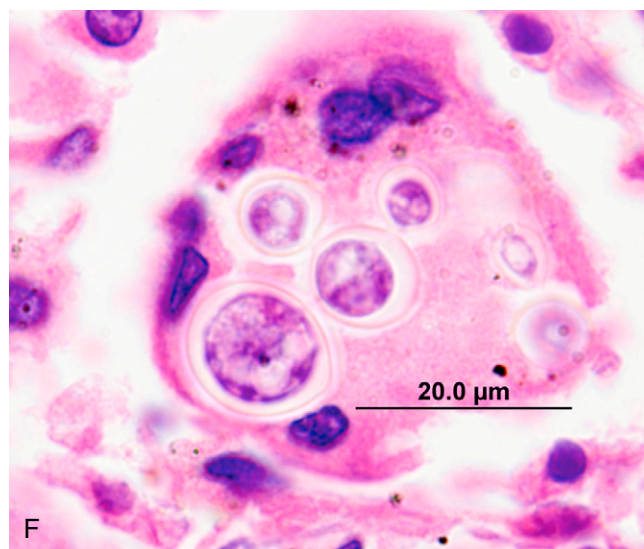
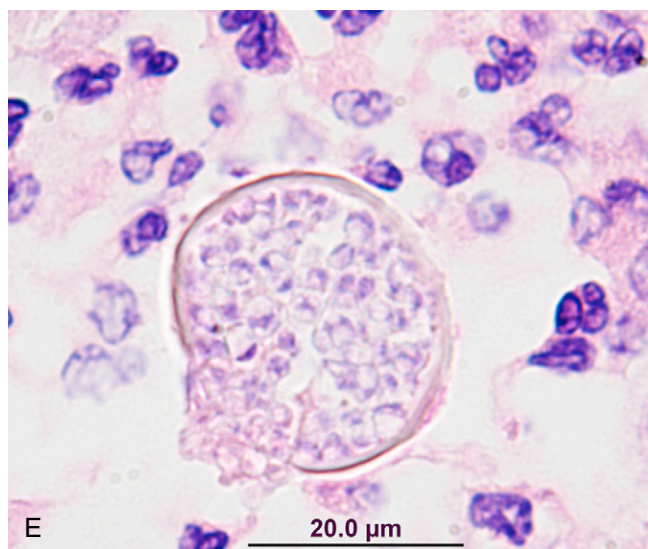
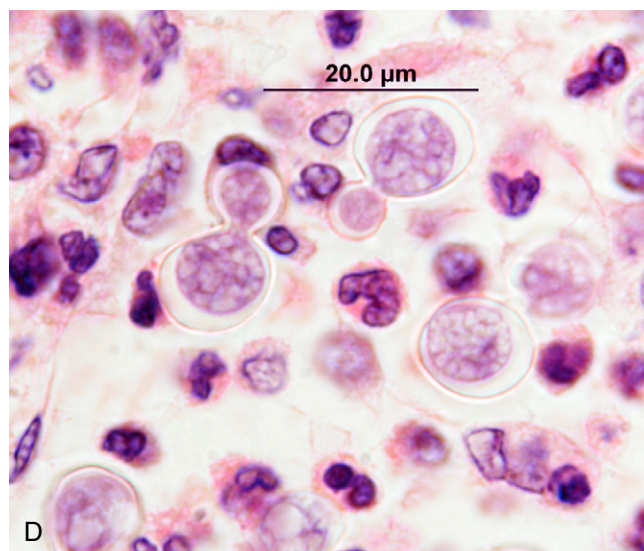
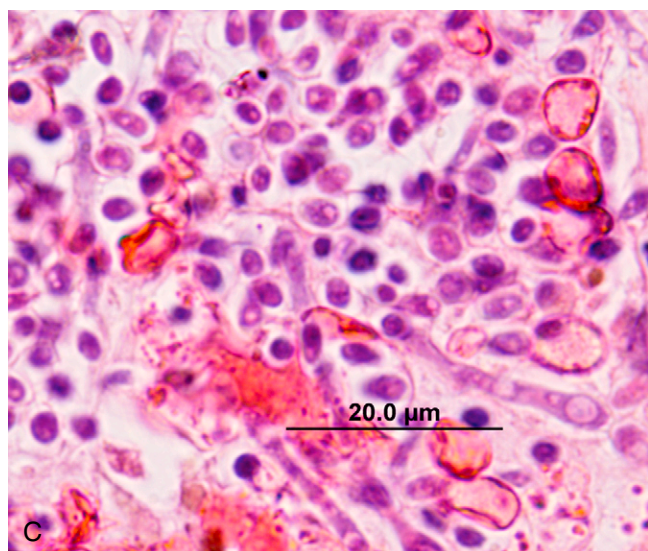
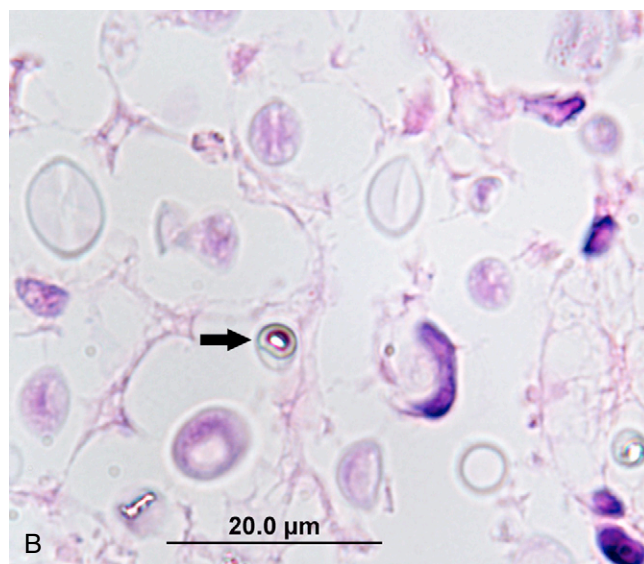
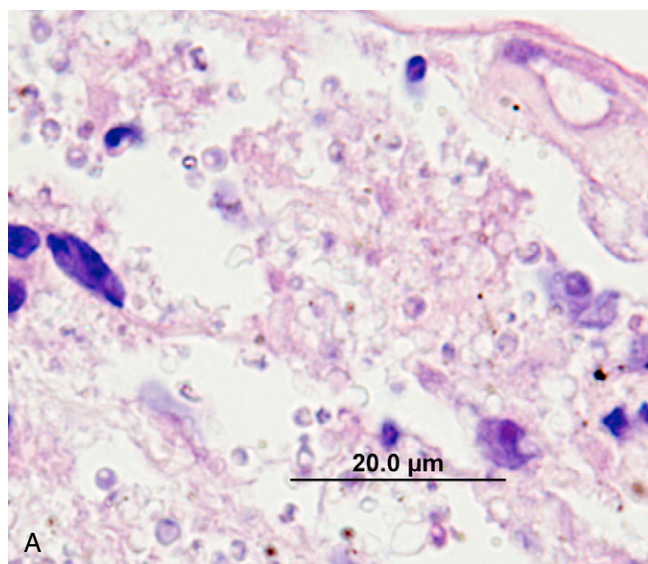
## Introduction

Both hematoxylin and eosin (H&E)<sup>1,2</sup> and Papanicolaou are excellent colorants for identification of fungi in tissue. In one study, autofluorescence was most valuable for identification of *Coccidioides immitis*, *Candida* species, and *Aspergillus* species, whereas neither *Histoplasma capsulatum* nor the zygomycetes were autofluorescent.<sup>3</sup> Special stains<sup>2,4,5</sup> allow for the detection of virtually all fungi; PAS is often used for fungus diagnosis, PAS with hematoxylin counterstain (PAS-H) is an excellent alternative because it accentuates the fungal organisms and the inflammatory response, whereas GMS provides the best contrast. Stains that have been substituted for GMS or PAS are Gridley's stain and fluorescent techniques such as Calcofluor white, Congo red, Uvitex 2B, and perhaps Tinopal CBS-X.<sup>6-11</sup> Two techniques are relatively specific for *Cryptococcus neoformans*:<sup>1</sup> those that stain acid mucopolysaccharides (e.g., Mayer's mucicarmine, Alcian blue, and colloidal iron), and the Fontana-Masson stain.<sup>12</sup> The Fontana-Masson stain is useful for differentiation of capsule-deficient *C. neoformans* from *H. capsulatum* and *Blastomyces dermatitidis*.<sup>13,14</sup> The Gram-Weigert stain<sup>15,16</sup> is an excellent stain for Gram-positive bacteria like actinomycetes, and *Pneumocystis* cysts, *Candida* spp. and *Histoplasma*. Figures 5-1 and 5-2 provide comparative illustrations of some of the more commonly encountered fungal pathogens. Figure 5-3 illustrates several special stains for diagnosis of fungal disease. The type of tissue response to fungi depends on the host's immune status and the manner in which the fungus interacts with the host. Simply summarized below are the 5 main histopathologic types of host response to fungi.

1. *Non-invasive colonization of a preexisting cavity.* Non-invasive fungal colonization of a cavity is referred to as a fungus ball. Acute and chronic inflammation may be seen within the cavity wall and occasionally superficial mucosal erosion can occur; there is no fungal invasion into adjacent tissue. Common sites for fungus balls are the paranasal sinuses and old pulmonary cavities such as those seen in chronic tuberculosis (Fig. 5-4).
2. *Allergic mucin-producing, non-invasive fungal disease.* These represent allergic hypersensitivity reactions associated with elevated IgE levels, fungus-specific precipitins and production of allergic mucin (AM). AM is composed of a mixture of mucin, cellular debris, eosinophils and

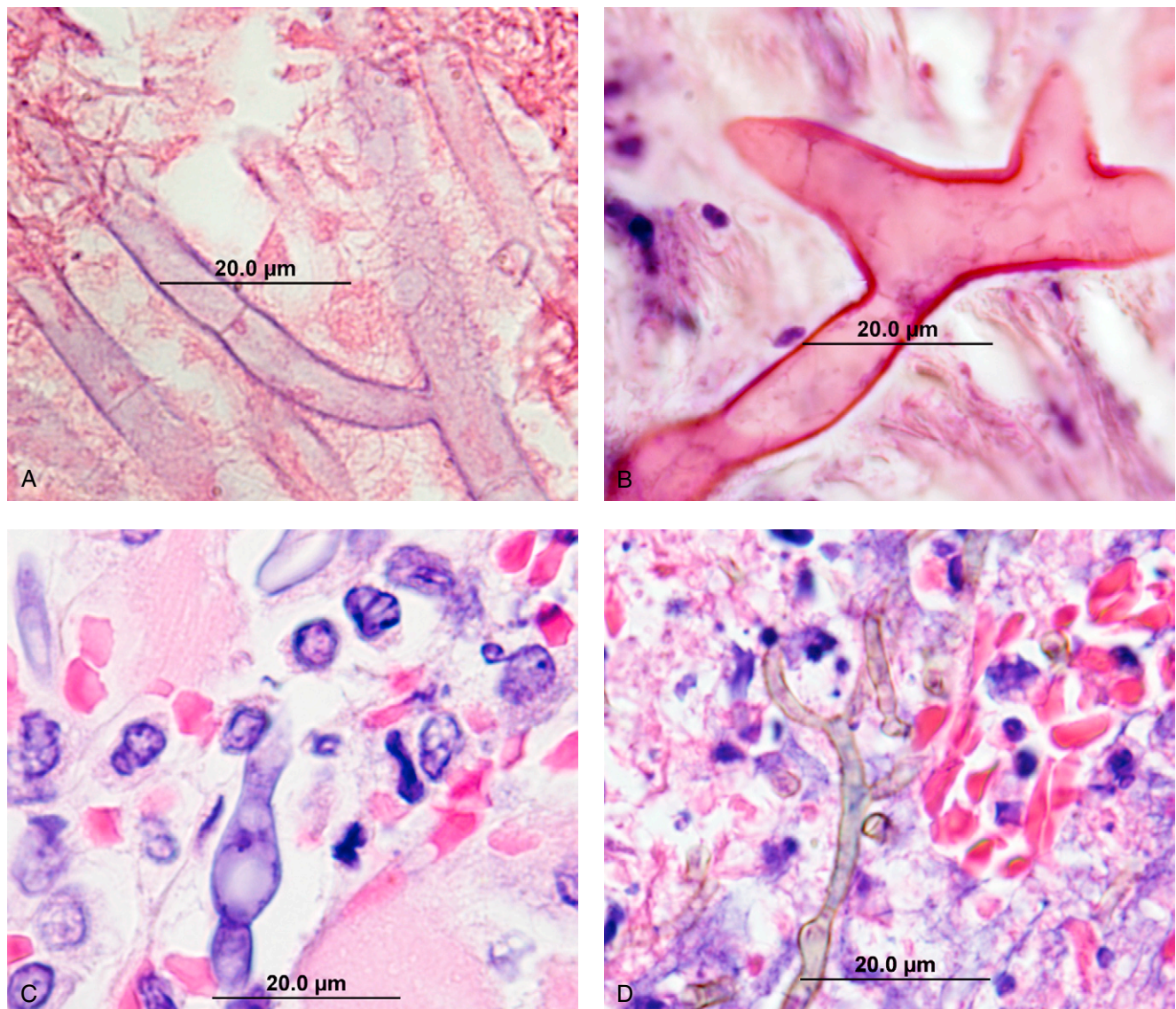
Charcot-Leyden crystals and may be found in fungal and non-fungal allergic disease, including asthma (Fig. 5-5).<sup>17</sup>

3. *Predominantly neutrophilic inflammatory response.* Patients with mild neutrophilic impairment may develop localized infections, and severely neutropenic patients are at risk for disseminated infection. Invasive infections are characterized by neutrophilic exudate and necrosis with liquefaction. Coagulative necrosis, without an associated inflammatory response, is seen in severely neutropenic patients. Fungal vascular invasion is typically found in severe infections (Fig. 5-6).
4. *Granulomata versus diffuse macrophage infiltration.* In patients with localized granuloma formation, extensive necrotizing granuloma formation and diffuse macrophage infiltration, the following may occur: small localized granulomata; extensive granuloma formation often with caseation, cavitation and fibrosis; or marked macrophage response without epithelioid transformation. Macrophages appear virtually engorged with intracytoplasmic organisms. The latter is found in AIDS and other severely T cell immunosuppressive conditions and should *not* be called granulomatous. Neutrophils are not typically seen in histologic or cytologic material from these types of infections unless there is bacterial superinfection or extensive necrosis. Figure 5-7 demonstrates two contrasting reactions to *H. capsulatum*. The first is a caseating granuloma in a person who developed a solitary lung nodule (Fig. 5-7A, B). The second is a liver section and a bronchoalveolar lavage sample (Fig. 5-7C, D).
5. *Mixed granulomatous and purulent inflammation.* The host response is a mixture of epithelioid macrophages and neutrophils. In localized, controlled infections, the granulomatous reaction dominates and organisms are very scant. In more fulminant infections, one sees a predominance of neutrophils, and organisms are readily seen. In advanced AIDS, the granulomatous response is essentially absent, and one sees abundant organisms, variable numbers of neutrophils, and extensive necrosis. Most dimorphic fungi will produce this spectrum of disease. Mixed purulent and granulomatous inflammation is seen in infections with *Sporothrix schenckii* and dematiaceous fungal infections. Figure 5-8A, taken from the resection of a solitary pulmonary nodule, illustrates localized blastomycosis. Figure 5-8B is lung tissue from a fatal case of fulminant pulmonary blastomycosis.



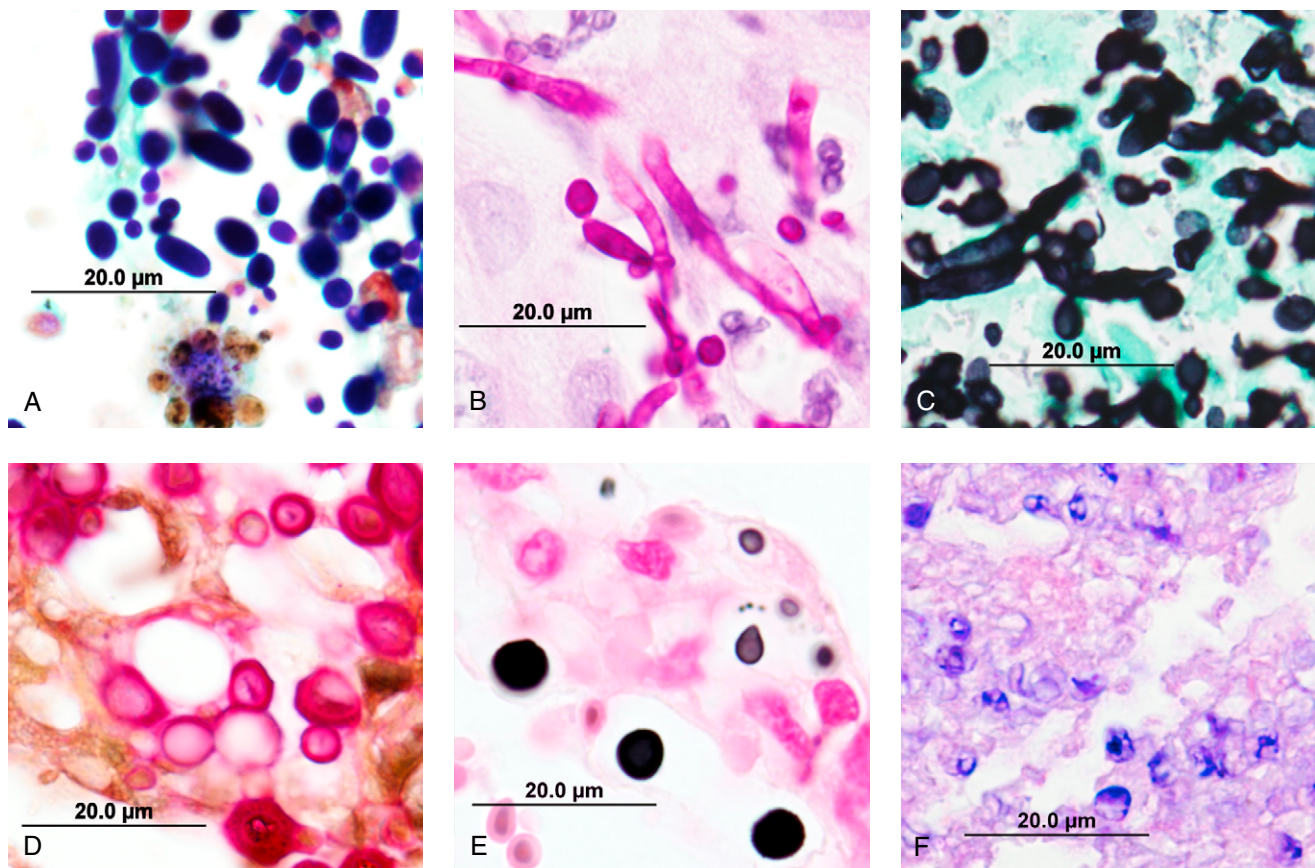


**Figure 5-1** (see opposite page) (A) *Histoplasma capsulatum* in lung. Yeasts are small and intracellular, with clear narrow halos. (B) *Cryptococcus neoformans* in brain. Yeasts are round, variable in size, appearing clear to pale blue, surrounded by clear spaces. Note the refractile granule (arrow) within one yeast cell. (C) *Candida albicans*, rectum. Yeasts are small, slightly larger than *Histoplasma*. Both blastoconidia and pseudohyphae are present. (D) *Blastomyces dermatitidis* in lung. Yeasts are oval with thick double-contoured cell walls and broad-based budding. (E) *Coccidioides immitis* in lung. Large, round spherule with refractile, double-contoured wall and small endospores. (F) *Paracoccidioides brasiliensis* in lung. Yeasts are round, variable in size, with double-contoured cell walls (H&E).

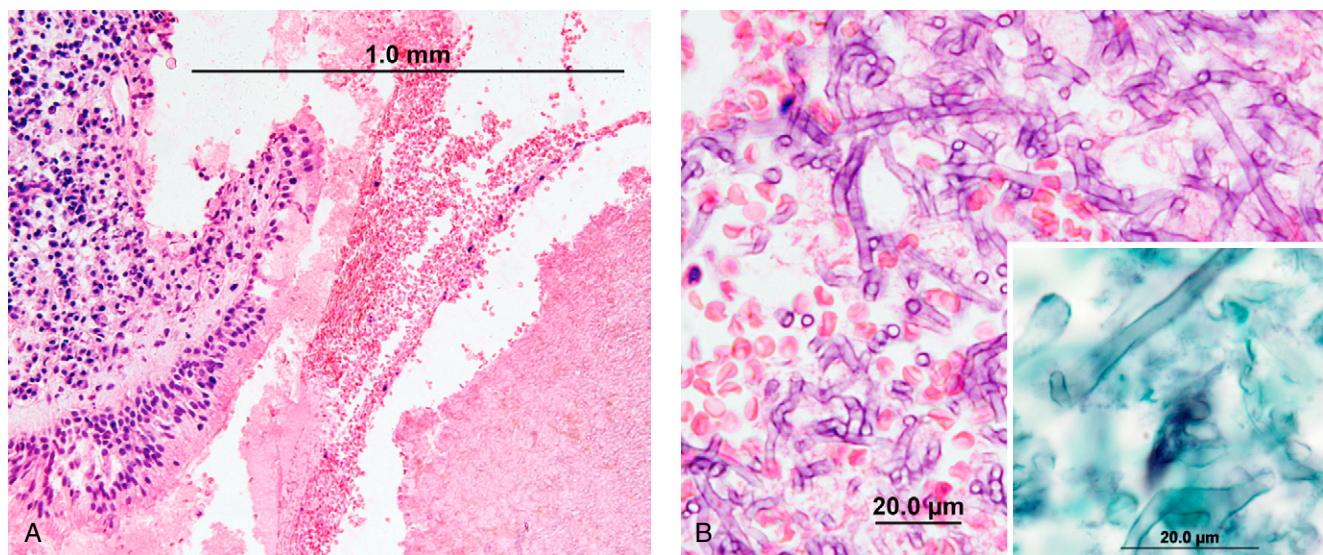


**Figure 5-2** (A) *Aspergillus* sp. in lung. Septate and dichotomous branching hyphae. (B) Zygomycete in soft tissue of paranasal sinuses. Dense eosinophilic staining, broad hyphae, with irregular branching. (C) *Fusarium* sp. in subcutaneous tissue. Irregularly swollen hyphae. (D) Dematiaceous fungus, cerebral phaeohyphomycosis. Although the fungus cannot be identified, its dematiaceous nature can be determined on the basis of the brown-staining hyphal wall (H&E).





**Figure 5-3** (A–C) *Candida albicans* in tissue stained by Gram (Brown-Hopps), periodic acid-Schiff/ hematoxylin (PAS-H), and Grocott methenamine silver (GMS) methods, respectively. (A) Dark blue, dense staining of yeasts and pseudohyphae. (B) Yeasts and pseudohyphae stain pink. The hematoxylin counterstain allows one to see the polymorphonuclear cell reaction (C) Yeasts and pseudohyphae stain dense black. The fungi appear larger owing to precipitation of silver around the fungal cell wall. (D) *Cryptococcus neoformans* stained by Mayer's mucicarmine. Bright red staining of cell wall with fainter staining of capsule. (E) *Cryptococcus neoformans* in Fontana-Masson melanin stain. Yeasts stained dense black. (F) *Pneumocystis jiroveci* in lung stained by Gram–Weigert method. Cyst walls are colored bright blue against a red background. Dark blue dots represent thickenings in cyst wall.



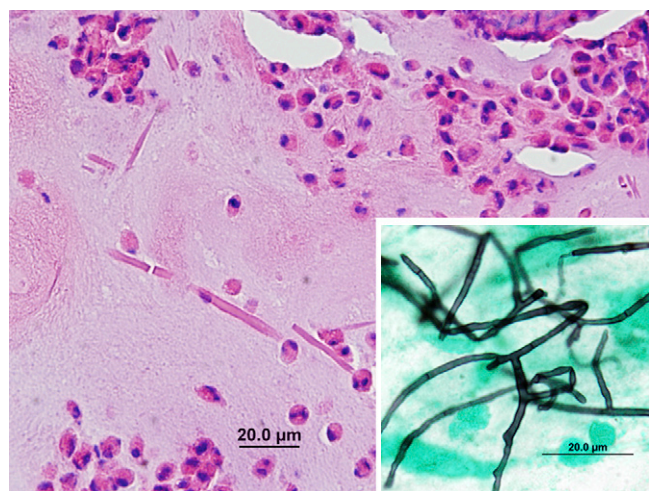
**Figure 5-4** Non-invasive fungus ball. (A) Hyphae are seen within paranasal sinus cavity. Some chronic inflammation without fungal invasion is seen within the sinus wall (H&E). (B) Hyphae within the sinus lumen. (H&E). Insert: Cytologic preparation of aspiration of sinus fungus ball (GMS).



## Yeast infections

### *Candida* spp.

In superficial candidiasis, yeasts and pseudohyphae are typically localized to the superficial squamous epithelium and often are not seen in biopsy samples owing to loss of exudate during processing. Lymphoplasmacytic inflammation and vascular congestion may be seen in the underlying submucosa and neutrophils within the epithelium.<sup>18-20</sup> In vaginal candidiasis, yeasts and pseudohyphae are entangled with clumps of squamous epithelial cells and variable numbers of neutrophils. The fungi sometimes appear to transfix stacks of squamous cells forming structures commonly known as “shish kebabs”<sup>21</sup>

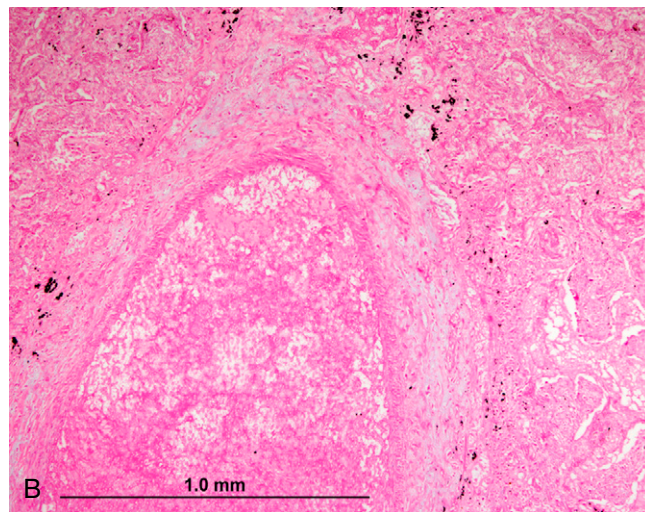
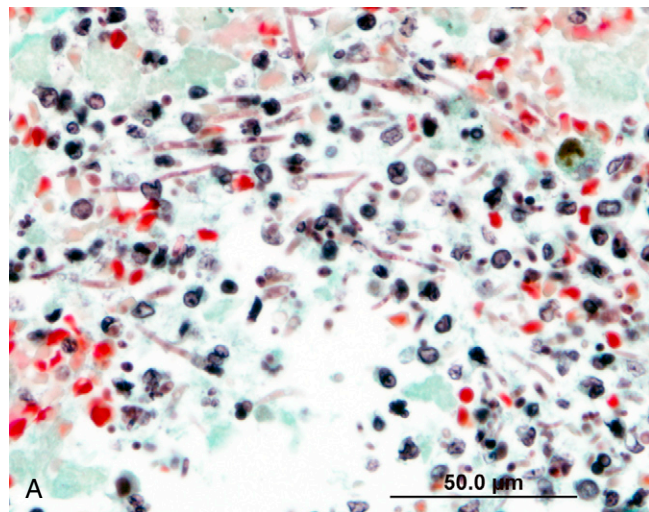


**Figure 5-5** Muroid impaction associated with fungi. Mucin, eosinophils and Charcot-Leyden crystals (H&E). Hyphae are in insert (GMS).

(Fig. 5-9). In the oropharynx, esophagus, and gastrointestinal tract, *Candida* colonization (Fig. 5-10A) is typically limited to the superficial portions of the epithelium. In more severe cases, often markedly neutropenic patients, extensive candidiasis of the gastrointestinal tract may occur with invasion into the submucosa or muscularis. In invasive candidiasis of the digestive tract, mucosal ulceration and extensive pseudomembrane formation are common (Fig. 5-10B, C). Pseudomembranes consist of necrotic cellular debris, fibrin and varying numbers of neutrophils. Within the pseudomembrane and underlying mucosa are numerous fungal elements (Fig. 5-10D), consisting predominantly of pseudohyphae with some budding yeasts, 2–6 µm in diameter, and occasionally septate hyphae. Submucosal vascular invasion is common and can result in hematogenous dissemination (Fig. 5-11).

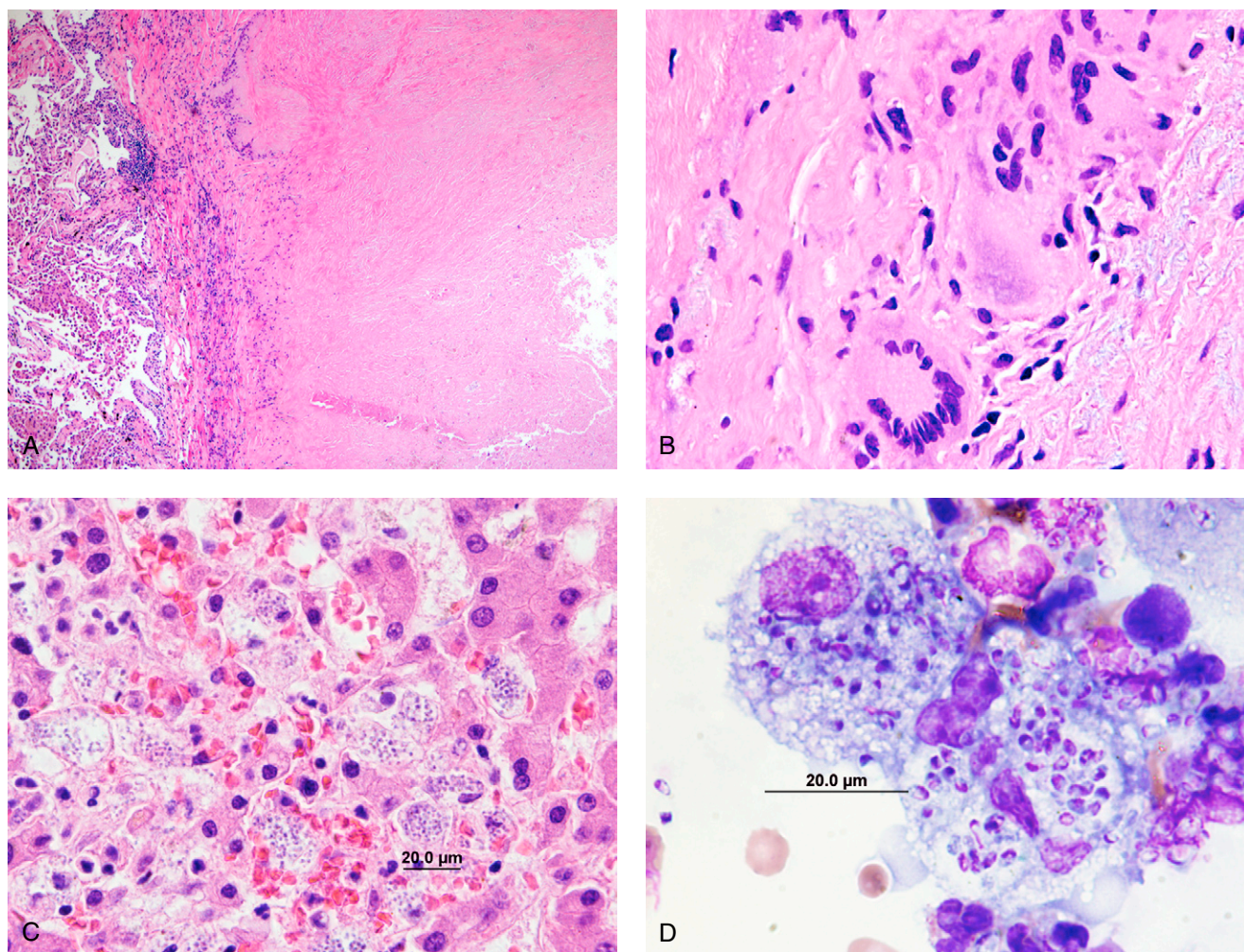
Neutrophilic reaction and necrosis are typical in patients who can muster a granulocytic reaction (see Fig. 5-6A). In the severely neutropenic patient, coagulative necrosis, hemorrhage and marked vascular invasion without neutrophils is typical, and intravascular thrombi may be found. Radiating growth of hyphae may also be seen<sup>22</sup> but granuloma formation is uncommon.<sup>18</sup> Caseating granulomata have been described in patients with previously treated hepatosplenic candidiasis.<sup>23</sup>

*Candida* is usually visible in H&E stained slides (see Fig. 5-1C), Gram, PAS and GMS stains in tissue (see Fig. 5-3A-C). The presence of oval, budding yeast, pseudohyphae, and true hyphae is characteristic of *Candida* species. The main differential for *Candida* in tissue is *Trichosporon*, a less common opportunistic fungus that is larger and forms arthroconidia. *Candida* can be confused with *H. capsulatum* and *Pneumocystis jiroveci* cysts (Fig. 5-12). *Histoplasma* is intracellular. *Pneumocystis* cysts are slightly larger and more spherical with frequent collapsed forms; additionally, they lack budding. *Cryptococcus* is much more variable in size and more globose than *Candida*.

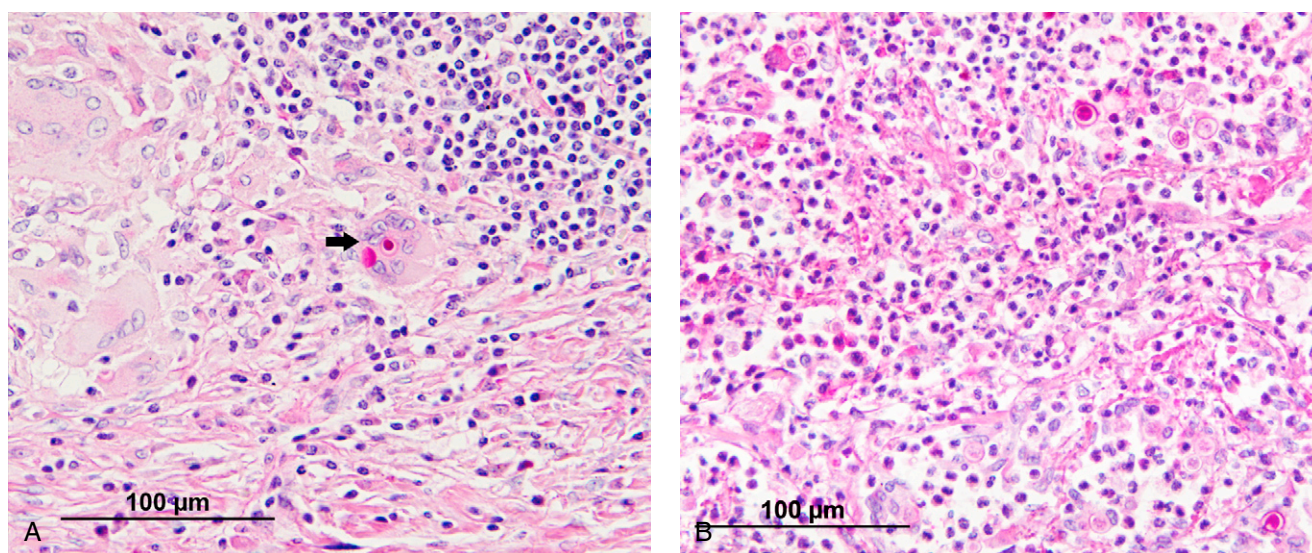


**Figure 5-6** (A) Invasive candidiasis in a patient with liver failure. Neutrophilic exudate and extravasated erythrocytes are admixed with pseudohyphae and blastoconidia (Movat stain). (B) Invasive aspergillosis in a severely neutropenic patient. Vascular thrombosis and marked and extensive coagulative necrosis with absence of neutrophilic response (H&E).



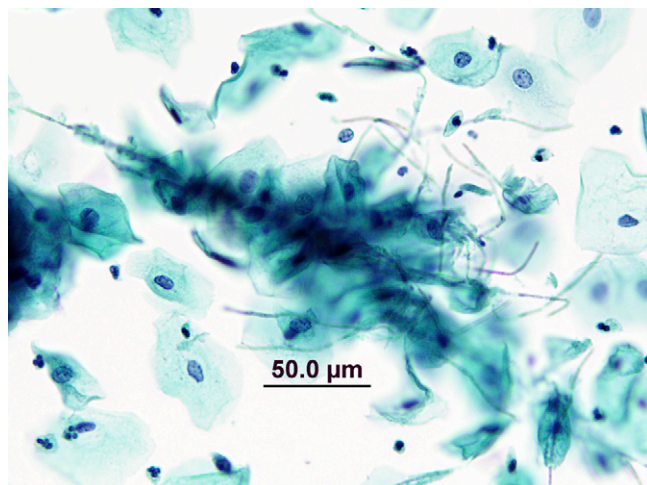


**Figure 5-7** Granuloma formation versus diffuse macrophage reaction in histoplasmosis. (A) Lung shows presence of well-formed, circumscribed granuloma, and central necrosis (H&E). (B) In the wall of the granuloma are epithelioid macrophages, giant cells and fibroblasts (H&E). (C) Section of liver showing massive enlargement of Kupffer cells that are filled with yeasts. Granulomata are absent (H&E). (D) Bronchoalveolar lavage from AIDS patient containing alveolar macrophages engorged with yeasts (Romanowski stain).

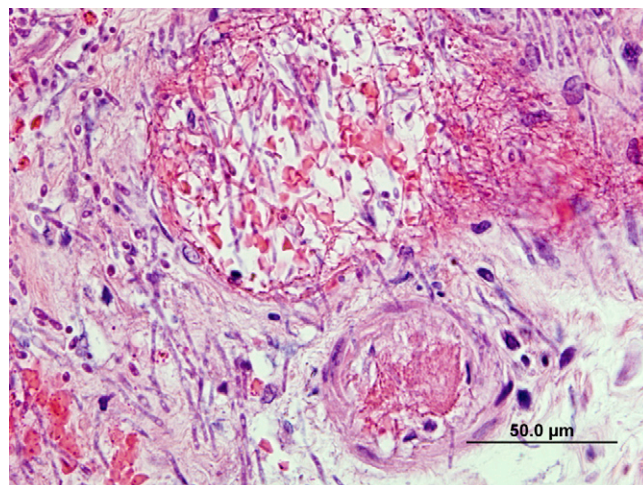


**Figure 5-8** (A) Localized blastomycosis. Granulomatous lesion containing epithelioid macrophages and giant cells surrounded by a rim of lymphocytes and plasma cells. One macrophage contains yeast (*arrow*) (PAS-H). (B) Fulminant blastomycosis. Alveolae are filled with neutrophils and abundant yeasts. Epithelioid macrophages are sparse (PAS-H).

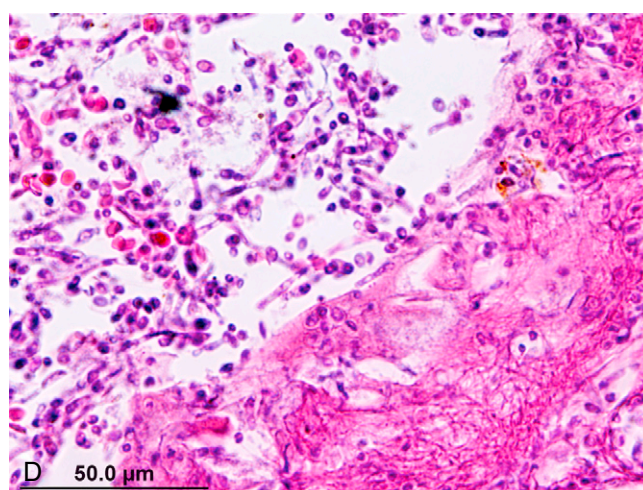
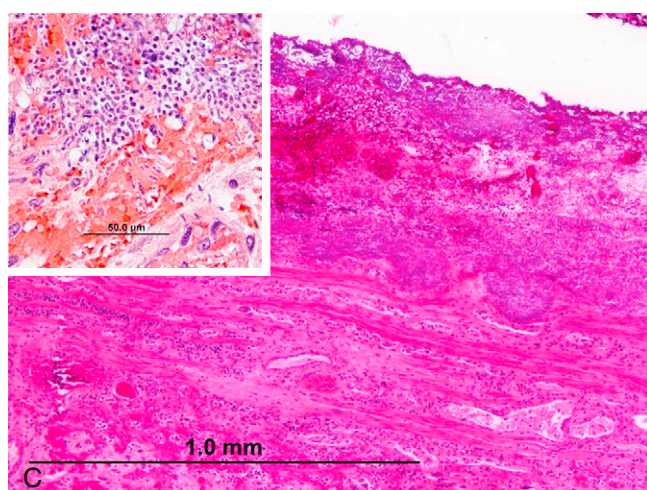




**Figure 5-9** *Candida* vaginitis in a liquid-based Papanicolaou test. Pseudohyphae transfix squamous epithelial cells (Papanicolaou stain).



**Figure 5-11** *Candida albicans* proctitis with presence of vascular invasion, intravascular fibrin and hemorrhage. Note predominance of pseudohyphae (H&E).



**Figure 5-10** (A) Endoscopic view of *Candida* esophagitis showing circumscribed, white mucosal plaques (photograph courtesy of Dr Manoop Bhutani). (B) Severe pseudomembranous, ulcerative esophagitis in a markedly neutropenic patient. Esophageal mucosa covered by a greenish exudate (reproduced from Archives of Pathology and Laboratory Medicine). (C) Esophagus seen in (B). The esophageal epithelium has been replaced by a dense, bluish-colored pseudomembrane (H&E). There is superficial invasion of the submucosa by fungi in insert (H&E). (D) Pseudomembrane consists of fungi, fibrin and some erythrocytes (H&E).



### *Cryptococcus neoformans*

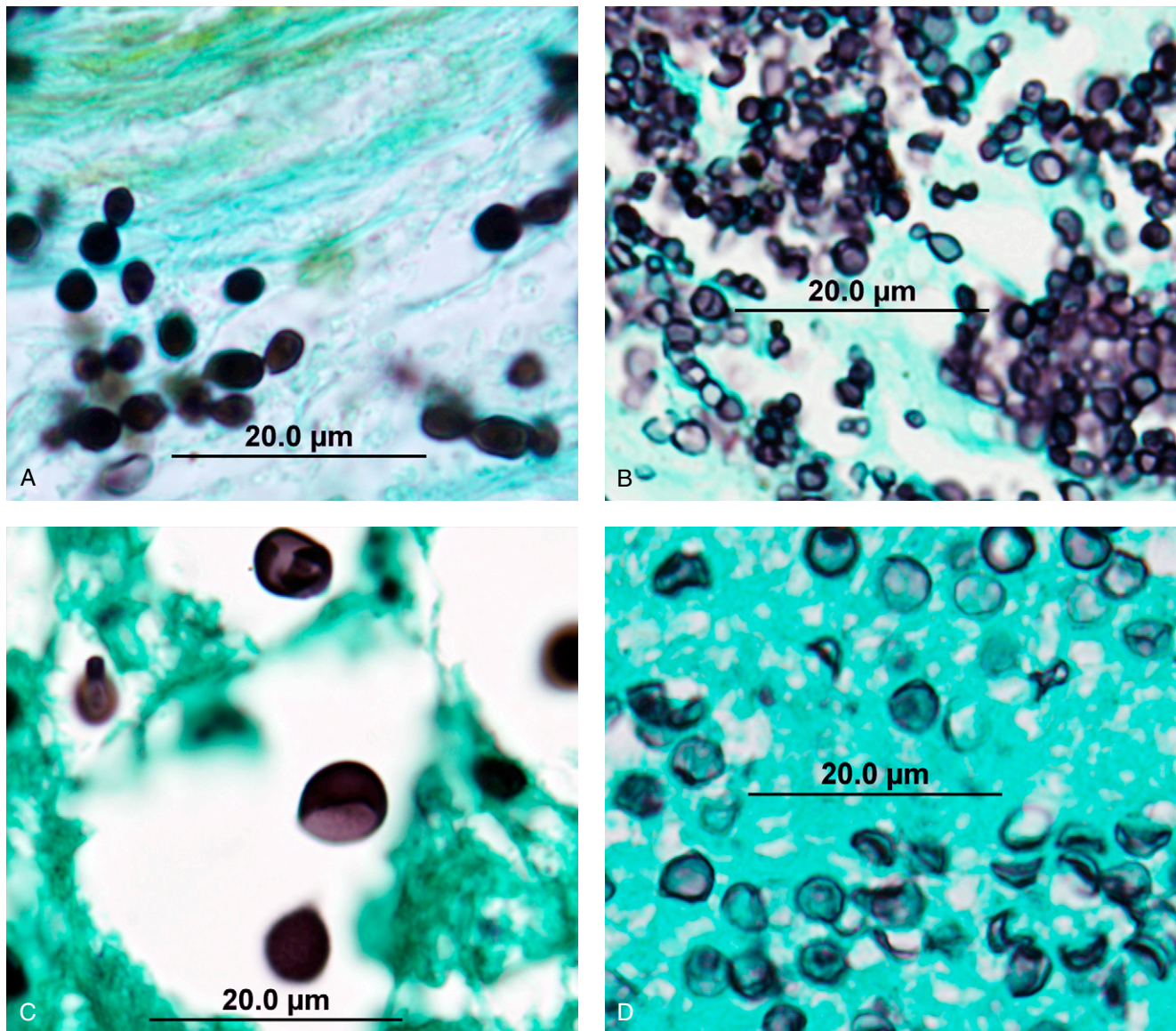
The host reaction to *Cryptococcus* varies with the immune status of the individual and the amount of cryptococcal capsule.<sup>24-26</sup> In chronic, localized infections, well-formed granulomata are typical. In contrast, in disseminated infection there is minimal inflammation, although scattered macrophages usually are present. Neutrophils may respond to cryptococcal infection; however, they are rarely seen in histologic sections in either form of cryptococcosis.<sup>24,25,27,28</sup> Presence of neutrophils in association with cryptococcosis may suggest bacterial superinfection or a response to massive necrosis. Neutrophilic response has been reported in sections from a case of infection with a capsule-deficient strain of *Cryptococcus*.<sup>26</sup>

In chronic granulomatous cryptococcosis, granulomata may be found in the lung, brain and meninges (Fig. 5-13), clinically mimicking neoplasia. In cytologic preparations from patients with localized or limited granulomatous disease,

macrophages often appear laden with circular structures that can be mistaken for either red blood cells or lipid. Their circular shape, variation in size, and capsules distinguish *Cryptococcus* from erythrocytes and other pathogenic fungi (Fig. 5-14). Centrally located refractile granules help differentiate *Cryptococcus* from lipid vacuoles (see Fig. 5-1B).

In severely T cell immunocompromised patients, granulomata are not formed. Yeast may continue to proliferate, resulting in an abundance of intracellular and extracellular growth that displaces normal tissue. This massive proliferation of encapsulated yeast and paucity of reactive mononuclear inflammatory cells creates multiple, yeast-filled lacunae in the infected tissue known as 'soap bubble lesions' (Fig. 5-15). In some severely T cell immunocompromised patients, there is widespread vascular dissemination of the fungus.<sup>29</sup>

Pulmonary cryptococcosis in AIDS patients, or otherwise severely T cell immunosuppressed patients, may manifest as either massive alveolar and interstitial or predominantly interstitial



**Figure 5-12** (A) *Candida* spp. (B) *Histoplasma capsulatum*. (C) *Cryptococcus neoformans*. (D) *Pneumocystis jiroveci* cysts (GMS).

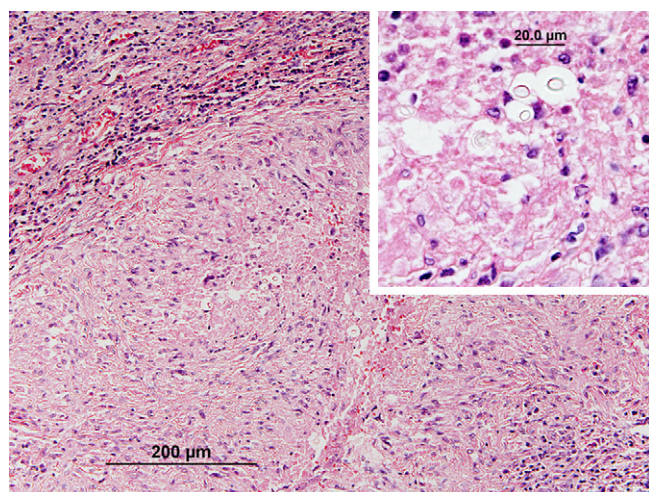


(intracapillary) infiltrates. Intraalveolar cryptococcosis may be associated with either an abundant macrophage reaction or little to no inflammatory response with marked expansion of alveoli by proliferating organisms.<sup>25,30</sup> A predominantly intracapillary and interstitial distribution of organisms may occur and be associated with widespread hematogenous dissemination (Fig. 5-16). Ulcerative or nodular skin lesion skin lesions (Fig. 5-17) may occur.

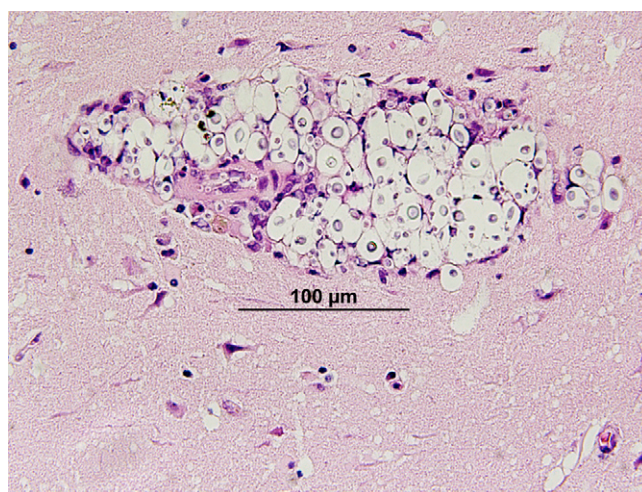
In tissue, *C. neoformans* appears as clear to pale blue, thin-walled, round to slightly oval yeast-like cells, often with a narrow, tube-like structure connecting the blastoconidia. Yeast cells vary in size from 2 to 20  $\mu\text{m}$  in diameter, but most are 4–10  $\mu\text{m}$ . Cryptococci may be difficult to visualize in an H&E-stained preparation (see Figs 5-13, 5-15). In H&E and GMS-stained sections, yeasts are typically surrounded by wide,

unstained mucinous capsules (see Fig. 5-12C). When there is massive yeast proliferation as in severely immunocompromised patients, pseudohyphae may be seen in tissue or cytologic preparations (Fig. 5-18A).

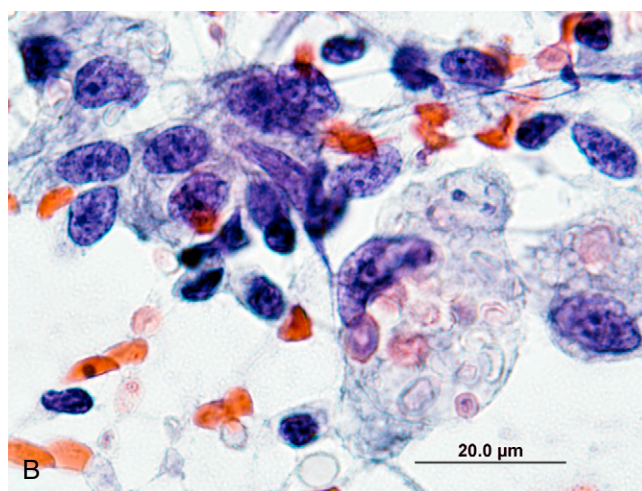
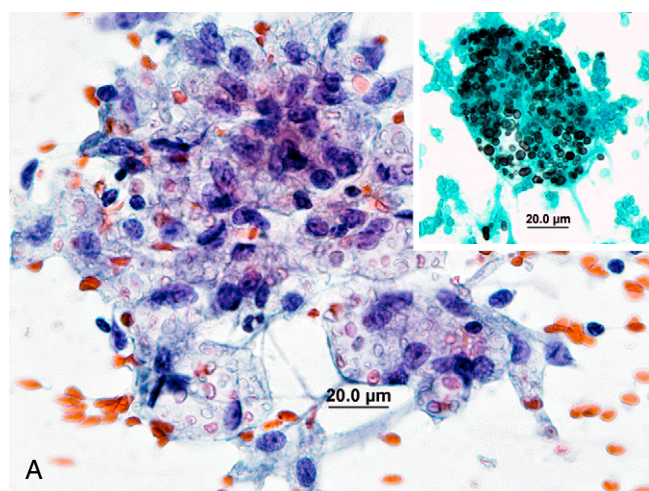
Mucicarmine stain colors the mucopolysaccharide of the cryptococcal cell wall and capsule (Fig. 5-18B). The cell walls of *B. dermatitidis* and *Rhinosporidium seeberi* are often weakly mucicarmine positive; however, their morphology is quite distinct from that of *Cryptococcus*. Capsule-deficient forms of *C. neoformans*, usually seen in immunocompetent hosts, may be difficult to find. Fontana-Masson staining is useful for finding capsule-deficient strains.<sup>13</sup> Melanin stain positivity (see Fig. 5-3E) permits one to differentiate *C. neoformans* from most other pathogenic yeasts. Exceptions are *Trichosporon beigelii*, which are also melanin positive.



**Figure 5-13** Well-formed cryptococcal granuloma that mimicked neoplasia in brain tissue. Nodular formation of epithelioid macrophages surrounded by a rim of fibroblasts, blood vessels and lymphocytes. Yeasts surrounded by clear non-staining capsules (H&E). Yeasts in insert (H&E).

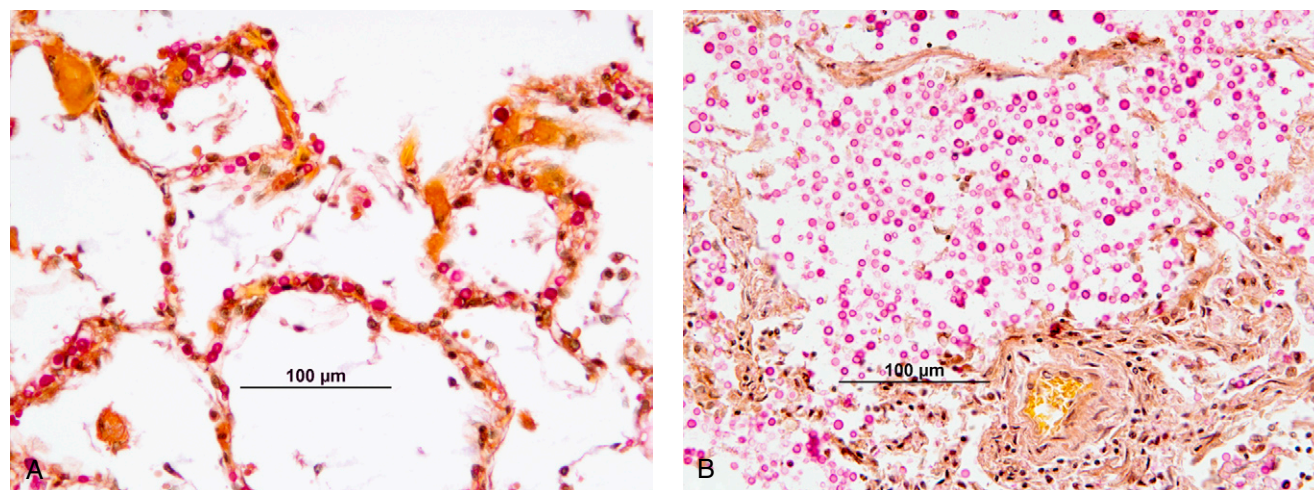


**Figure 5-15** Autopsy brain section from AIDS patient with cryptococcal meningoencephalitis. The yeast has expanded the brain tissue with minimal mononuclear cell response (H&E).

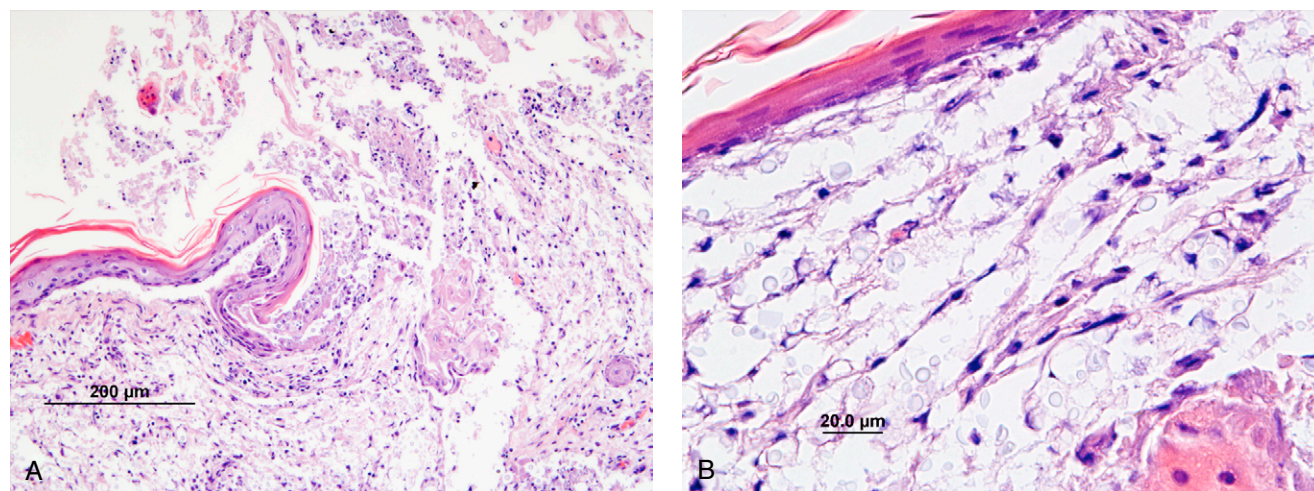


**Figure 5-14** Fine needle aspirate of lung with pulmonary nodules. (A,B) Views showing aggregates of vacuolated macrophages containing spherical structures that could be confused with red blood cells (Papanicolaou). Insert shows abundant intracellular yeasts (GMS).





**Figure 5-16** Intracapillary and intraalveolar patterns of pulmonary cryptococcosis. (A) Marked intracapillary infiltration of yeast with relatively few intraalveolar organisms (Mayer's mucicarmine). (B) Intraalveolar spread in a transplant patient. Expansion of alveolar spaces and destruction of alveolar walls (Mayer's mucicarmine).



**Figure 5-17** (A) Ulcerative skin lesion of cryptococcosis. Marked infiltration, and expansion of dermal soft tissue with no significant inflammatory response (H&E). (B) Pale blue yeasts are seen with absence of inflammatory reaction (H&E).

## Infections caused by dimorphic fungi

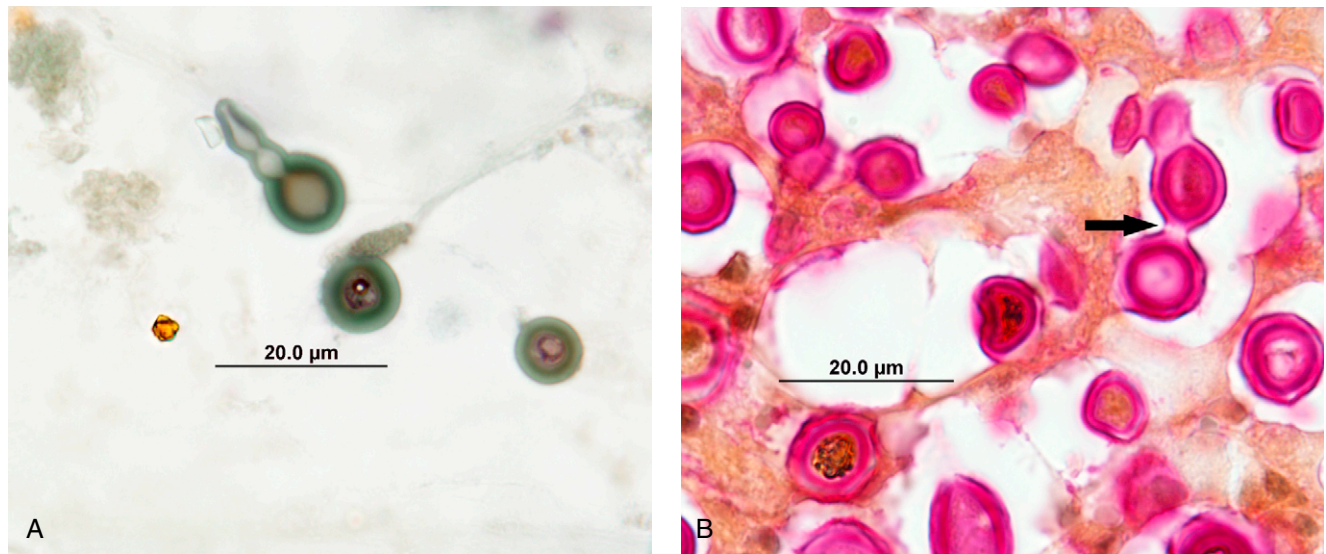
### *Histoplasma capsulatum*

Cell-mediated immunity is important in defense against and intracellular killing of *H. capsulatum*.<sup>31</sup> The spectrum of histopathologic findings ranges from localized granuloma formation to massive aggregates of non-activated macrophages containing myriad yeast forms with absence of granuloma formation.<sup>32</sup> The latter is a common histologic finding in AIDS patients whose macrophages permit intracellular replication of *H. capsulatum*.<sup>33</sup> Neutrophils are rarely seen in histologic or cytologic preparations from histoplasmosis unless there is massive necrosis or bacterial superinfection.

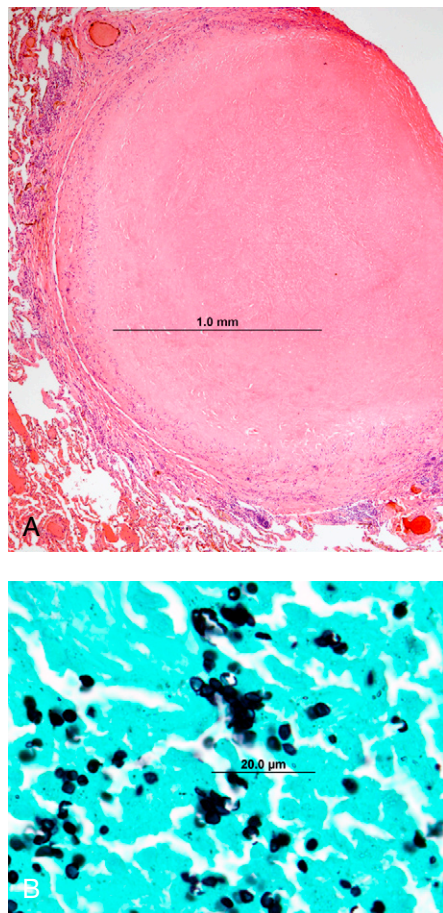
Early pulmonary lesions are characterized by aggregates of histiocytes within alveolar spaces. Expansion of these lesions

causes parenchymal necrosis, followed by granuloma formation. During the acute stage, lymphohematogenous dissemination is common, even in asymptomatic, immunocompetent individuals.<sup>32</sup> Residua of this event are multiple, small granulomata that are incidental findings during radiologic evaluation or autopsy. These lesions, which are often calcified, are commonly located in the pleura and spleen and are frequently the only indication of disseminated *H. capsulatum*. Although the lumina of these tiny granulomata are often completely replaced by hyalinized fibrous tissue, occasionally there is residual caseous necrosis in which yeasts can be seen. In some cases, massive pulmonary hilar and mediastinal lymph node calcification may occur and lead to lymph node erosion and broncho-lithiasis.<sup>34</sup> If organisms are found, they are often swollen and irregular in shape. Budding forms are often not identified, presumably owing to non-viability of these organisms (Fig. 5-19).

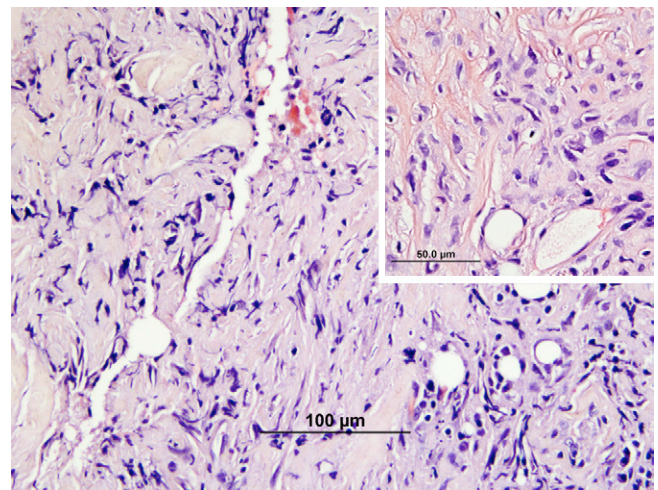




**Figure 5-18** (A) Cytologic preparation of sputum from AIDS patient with disseminated cryptococcosis. Pseudohyphae with centrally located refractile granules (Papanicolaou). (B) Brain with cryptococcal meningoencephalitis. Yeast cells are connected by a narrow, tubular structure (arrow) (Mayer's mucicarmine).



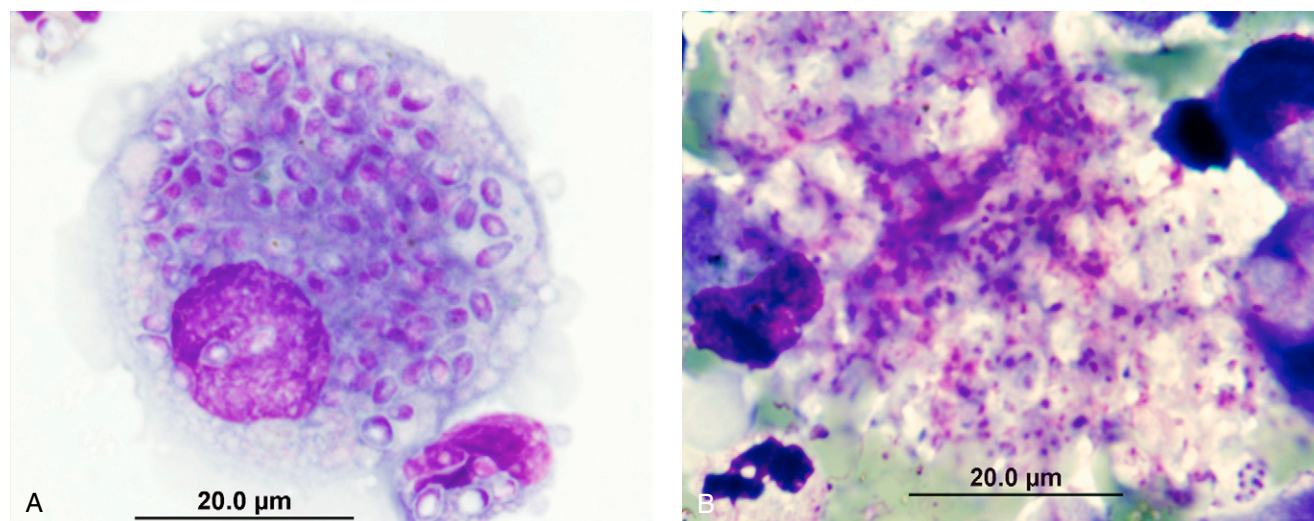
**Figure 5-19** Subpleural granuloma containing yeasts consistent with *H. capsulatum*. (A) A partially hyalinized granuloma surrounded by a narrow rim of fibrous tissue and mononuclear cells with residual central caseation (H&E). (B) Distorted, swollen, small yeasts are seen near the center of the granuloma (GMS).



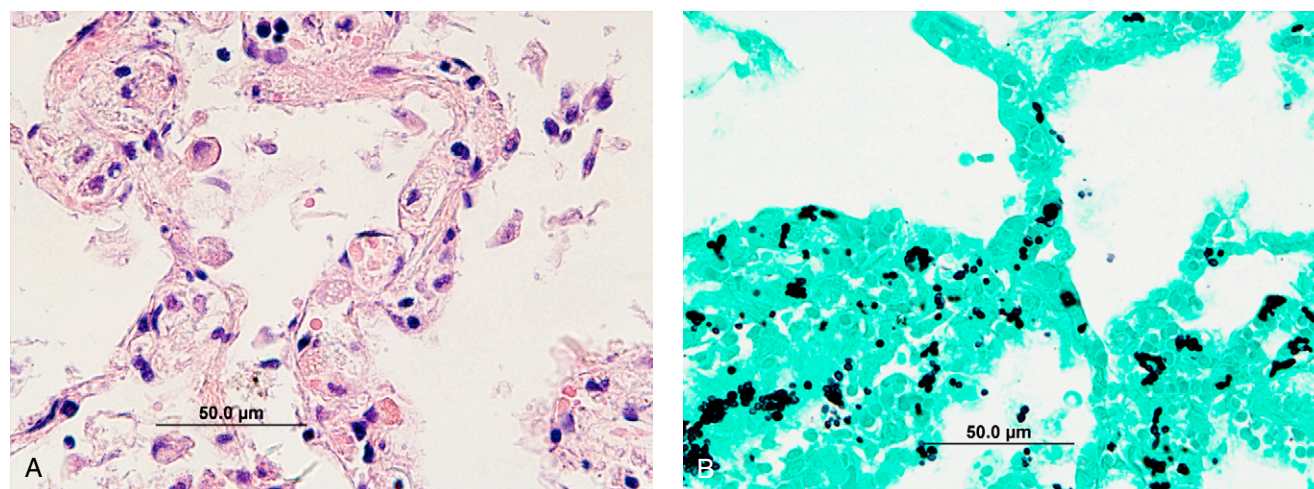
**Figure 5-20** Mediastinal biopsy of sclerosing mediastinitis resulting in superior vena cava syndrome. Dense fibrosis admixed with macrophages and lymphocytes. Neither caseating granulomata nor organisms were found (H&E). Insert: macrophages, fibrosis and capillary blood vessels (H&E).

In mildly immune compromised individuals, a chronic necrotizing form of the disease resembling fibrocaseous tuberculosis may be found. Fibrosis, granuloma formation with caseous necrosis and hilar lymphadenopathy are typical. Compared to tuberculosis, chronic histoplasmosis tends to cause more enlargement and heavier calcification of hilar lymph nodes with presence of laminated layers of fibrosis. Ossification within the calcified regions has been described.<sup>34</sup> An unusual complication of *H. capsulatum* infection is fibrosing mediastinitis, an immunologically mediated disease believed to be related to an aggressive hypersensitivity reaction to the organism. This is characterized by excessive fibrous tissue deposition around granulomata within the mediastinum (Fig. 5-20). Fibrosis and





**Figure 5-21** Bronchoalveolar lavages from AIDS patients with histoplasmosis and *Pneumocystis*, respectively. (A) Histoplasmosis. An alveolar macrophage with small, oval yeast cells having clear halos representing a cytoplasmic contraction artifact (Giemsa). (B) *Pneumocystis*. Aggregates of extracellular organisms having magenta/purple-colored dot-like nuclei and bluish cytoplasm. Cysts appear as non-staining, clear circles.



**Figure 5-22** Pulmonary histoplasmosis in an AIDS patient. (A) Small, intracellular, refractile bodies are seen within the interstitium and alveolar capillaries (H&E). (B) Silver stain reveals abundant yeast (GMS).

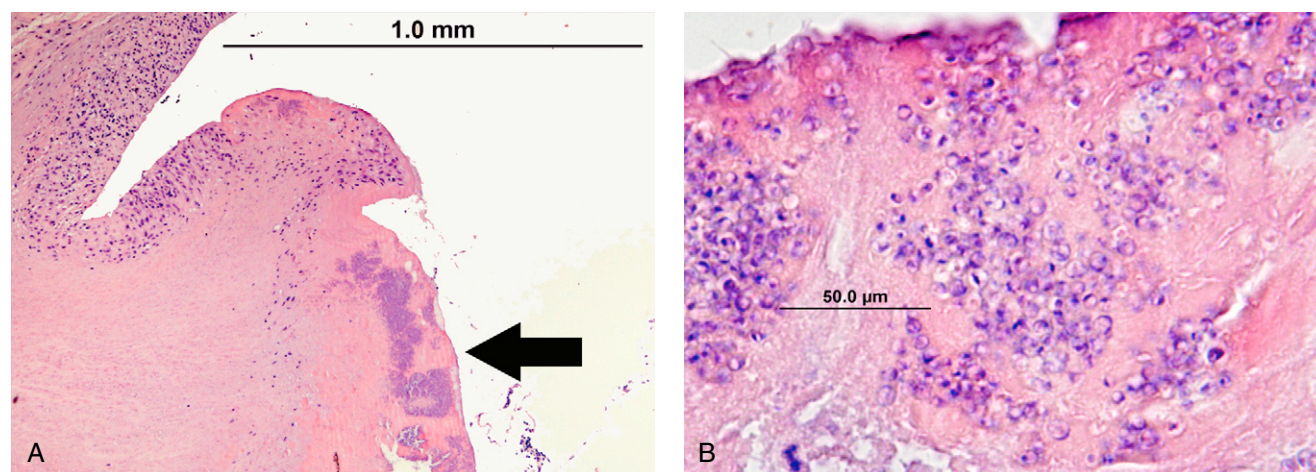
lymphoplasmacytic infiltration are common. Occasionally, residual granulomata, caseation and, rarely, stainable organisms are found.<sup>35-37</sup>

In severely T cell immunocompromised persons, particularly those with AIDS, infection with *H. capsulatum* can produce a fulminant infection, predominantly involving lungs and organs rich in mononuclear phagocytes. In these cases, there is marked proliferation of organisms within non-activated macrophages without granuloma formation. Yeast cells in disseminated histoplasmosis are most commonly found within macrophages. Exceptions are cases in which there is massive tissue necrosis. In this instance, extracellular yeast forms may be seen in abundance. In AIDS, histoplasmosis commonly presents with diffuse interstitial or reticulonodular pulmonary infiltrates.<sup>38</sup> In diagnostic bronchoalveolar lavage samples, *Histoplasma* yeast forms can be distinguished from *P. jiroveci*

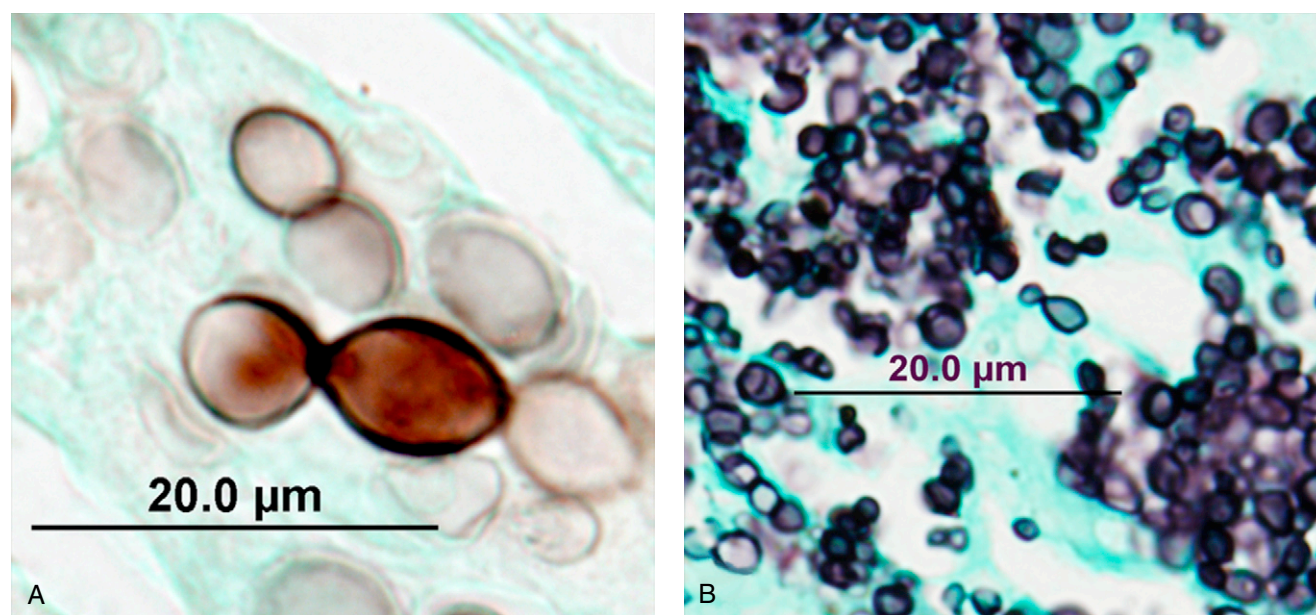
on the basis of their intracellular location, their smaller size, oval shape, and the presence of budding (see Figs 5-7, 5-12). Romanowski stains are excellent colorants for distinguishing between *H. capsulatum* and *Pneumocystis* in cytologic preparations (Fig. 5-21A). Only the intracystic and extracystic bodies of *Pneumocystis* are stained by the Romanowski technique. The cyst wall appears as a clear circle enclosing up to eight intracystic forms (Fig. 5-21B).

Disseminated histoplasmosis is characterized by aggregates of non-transformed macrophages. Myriad yeasts are found within mononuclear phagocyte system cells, including pulmonary alveolar macrophages, hepatic Kupffer cells and in lymph nodal, splenic and bone marrow macrophages (see Fig. 5-7C, D). In the lungs, intracellular yeast may be found within the interstitium, alveolar capillaries and the alveolar lumina (Fig. 5-22). Massive adrenal necrosis with subsequent





**Figure 5-23** *Histoplasma* endocarditis, aortic valve vegetation. (A) Aggregates of blue (arrow) indicating focus of abundant yeasts against a red-staining, amorphous background (H&E). (B) Yeasts surrounded by narrow, clear halos are entrapped in fibrin. Absence of significant inflammatory cell reaction (H&E).



**Figure 5-24** Comparative features of *H. capsulatum* var. *duboisii* and *H. capsulatum* var. *capsulatum* in GMS. (A) var. *duboisii*. Yeasts are larger with thicker cell walls, and pseudohyphae (GMS). (B) var. *capsulatum*. Smaller oval yeasts (GMS).

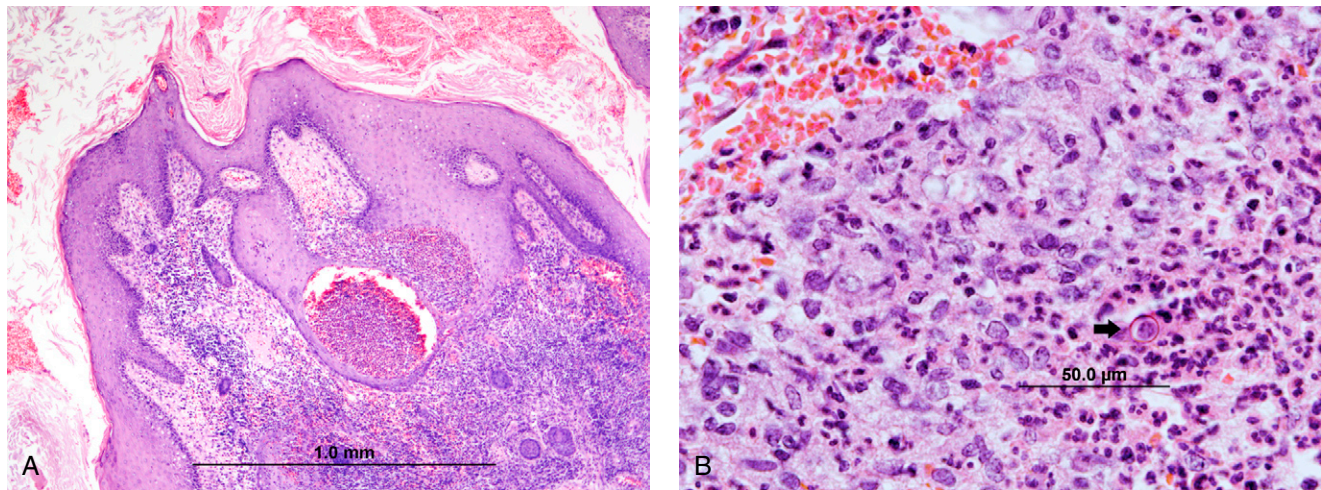
Addison disease secondary to *Histoplasma* adrenal vasculitis is not uncommon in disseminated infections.<sup>32</sup>

*Histoplasma* endocarditis is an exception to the rule that disseminated histoplasmosis is characterized by an infiltrate of macrophages with intracellular yeast forms.<sup>32,39</sup> In this disease, abundant fungal organisms are enmeshed within a non-destructive, fibrinous exudate. Calcifications may be found; however, neither mononuclear inflammatory cells nor necrosis is common (Fig. 5-23). There has been at least one case report of endocarditis caused by *H. capsulatum* in which sections of the vegetation showed bizarre, giant yeast-like forms and pseudohyphae.<sup>40</sup>

The histologic appearance of yeast cells of *H. capsulatum* var. *capsulatum* can be confused with *Penicillium marneffei*. *P. marneffei* reproduces by fission, forming a single transverse septum. Yeast cells of *H. capsulatum* also may be confused with intracellular amastigotes of *Leishmania* spp. and *Trypanosoma cruzi*; however, these protozoans have small, bar-shaped kinetoplasts that are visible in H&E-stained sections, but are best seen in sections stained by a reticulum or Romanowski method. Immunohistochemistry also may be useful, particularly for identification of *P. jiroveci*.

*H. capsulatum* var. *duboisii* is 8–15 μm in diameter, considerably larger than *H. capsulatum* var. *capsulatum* (Fig. 5-24).





**Figure 5-25** Cutaneous blastomycosis. (A) Pseudoepitheliomatous hyperplasia, some hyperkeratosis and a prominent dermal inflammatory infiltrate. An epidermal microabscess is seen (H&E). (B) Dermal infiltrate is a mixture of neutrophils and macrophages. A single yeast (arrow) (H&E).

This fungus generally elicits a granulomatous inflammatory response, commonly associated with numerous giant cells that may contain abundant phagocytized yeasts. Occasionally, caseation and a mild neutrophilic reaction may occur.<sup>22,41</sup> The most common manifestation of *H. capsulatum* var. *duboisii* infection is subcutaneous nodules; however, pulmonary and disseminated infections occur. Yeast cells of *H. capsulatum* var. *duboisii* are globose to oval, uninucleate, thick cell walled, and bud by a narrow base. History of residence in, or travel to, endemic regions of Africa should suggest this pathogen in the proper clinical setting.

### ***Coccidioides immitis*, *blastomyces dermatitidis* and *paracoccidioides brasiliensis***

In disseminated infections, secondary cutaneous lesions are common with *C. immitis* and *P. brasiliensis*, and secondary mucous membrane lesions also may occur. The histopathologic spectrum of these organisms ranges from predominance of epithelioid macrophages with localized disease to presence of myriad organisms, marked necrosis, variable numbers of neutrophils and absence of granuloma formation. The latter form is typically found in AIDS patients.<sup>42-44</sup> In immunocompetent persons, asymptomatic or isolated pulmonary lesions are common. All three of these fungal pathogens can readily be seen in H&E-stained tissue sections (see Fig. 5-1). Secondary cutaneous infections with these fungi show marked pseudoepitheliomatous hyperplasia. The dermis, however, contains an inflammatory infiltrate composed of epithelioid macrophages, giant cells and microabscesses. Neutrophilic microabscesses may also be found within the epidermis. This telltale combination of inflammatory cells is a signal to look for dimorphic fungi (Fig. 5-25).

#### ***Coccidioides immitis***

Inhaled arthroconidia enlarge and round up to form thick-walled, immature spherules that measure 5–30 μm in diameter in the lungs. As they reach maturity, spherules endosporulate by cleavage, forming endospores.<sup>41</sup> Mature spherules typically are 30–100 μm in diameter filled with 2–5 μm diameter, uninucleate endospores that contain punctate, PAS- and GMS-positive

cytoplasmic inclusions. When the spherule ruptures, thin-walled endospores are released into the surrounding tissue. Neutrophilic inflammatory reaction is typically seen predominantly around newly released endospores. Maturing spherules and old, empty spherules are often phagocytized by epithelioid and giant cell macrophages (Fig. 5-26).

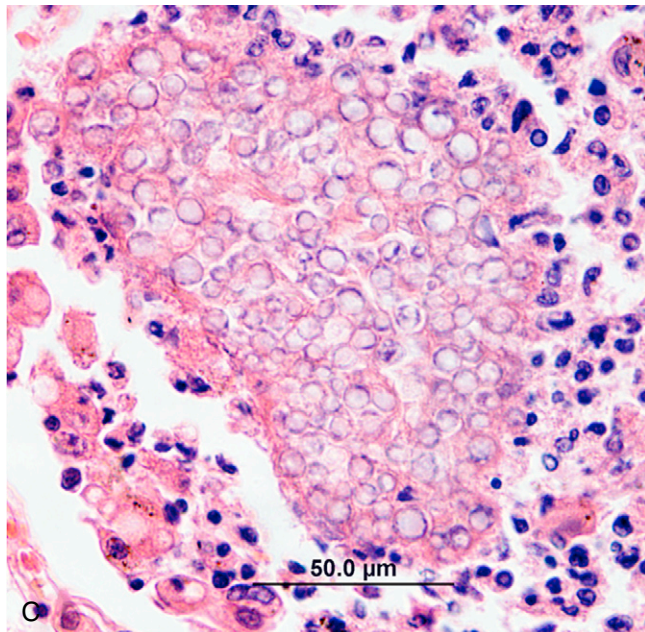
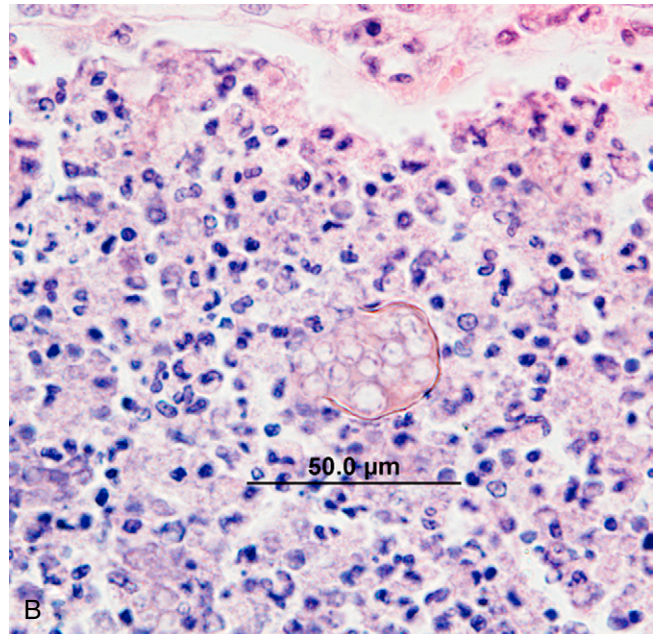
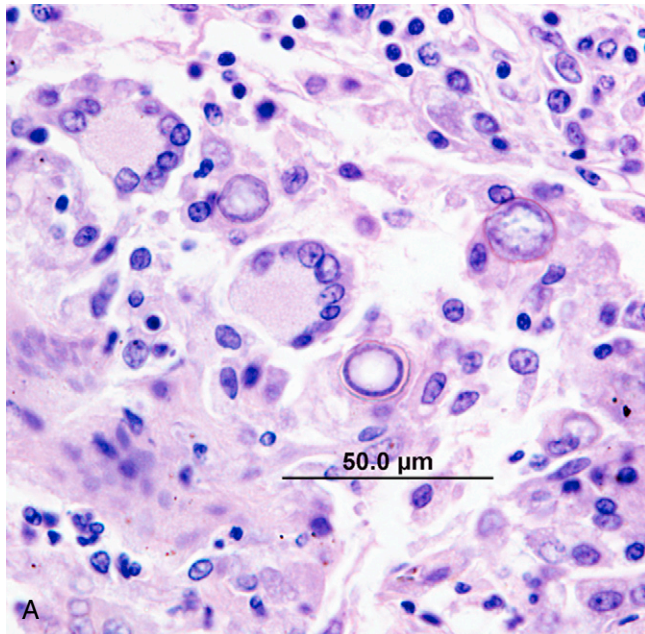
In heavy infections, as in AIDS, typically there is marked necrosis without granulomata, and mature and immature spherules. Immature spherules and recently released endospores can be mistaken for other fungi (Figs 5-27, 5-28). In contrast, spherules are sparse in older, fibrocaseous lesions, and often do not have the classic morphology. Marked peripheral blood eosinophilia and prominent eosinophil infiltration of tissue may accompany disseminated coccidioidomycosis.<sup>45,46</sup> Abundant eosinophils should suggest the possibility of *C. immitis* infection (Fig. 5-29).

#### ***Blastomyces dermatitidis***

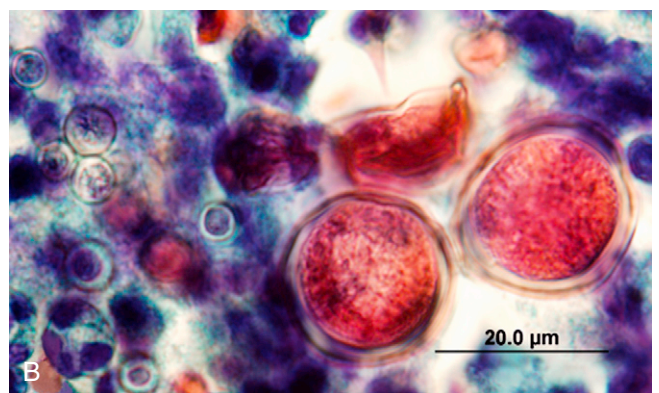
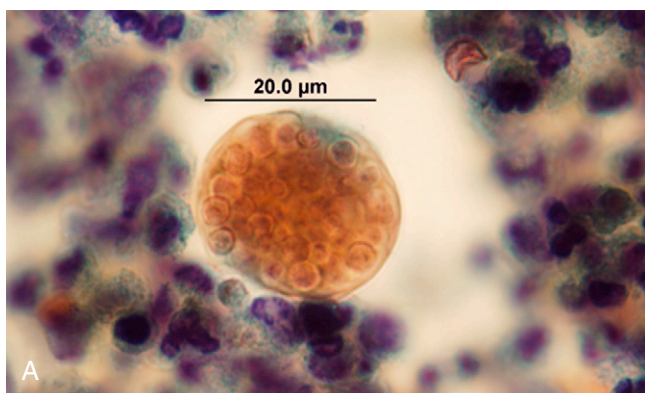
Comparative histologic features of *B. dermatitidis*, *C. immitis* and *P. brasiliensis* are illustrated in Figures 5-1D-F. Figure 5-8A is a localized granulomatous lesion that was misdiagnosed as a neoplasm. The inflammatory infiltrate consists predominantly of epithelioid macrophages with few neutrophils. *B. dermatitidis* was scanty, and best visualized with PAS-H. Figure 5-8B shows a fulminant bronchopneumonia caused by *B. dermatitidis*. Note the relative paucity of macrophages, abundance of neutrophils and many yeast cells of *B. dermatitidis*. Multinucleation is typical of *B. dermatitidis* but this feature may not be obvious. Multinucleation appears as tiny, hematoxyphilic dots (Fig. 5-30A) that are highlighted with PAS-H stain (Fig. 5-30B). Although the width of the bud attachment (broad-based) is the most useful diagnostic criterion for diagnosis of *B. dermatitidis*,<sup>41</sup> budding is not always present.

In tissue, yeast cells of *B. dermatitidis* are most likely to be confused with immature spherules of *C. immitis* or with *P. brasiliensis*. Capsule-deficient forms of *C. neoformans* can be differentiated on the basis of melanin staining. Atypical forms of *B. dermatitidis* infrequently encountered in tissue include yeast-like microforms measuring 2–4 μm in diameter, which may be confused with *H. capsulatum*, and hyphal or filamentous forms.<sup>47-51</sup>



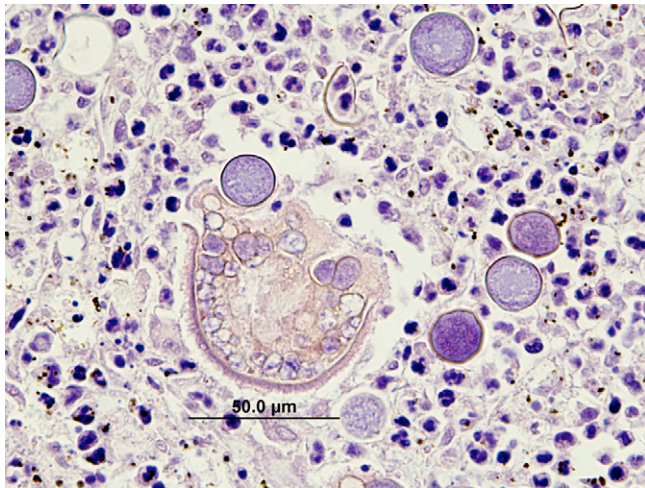


**Figure 5-26** Pulmonary coccidioidomycosis. (A) Epithelioid macrophages and giant cells surrounding and having phagocytized non-sporulating spherules. (B) Neutrophilic exudate surrounds rupturing spherule. (C) Extracellular, immature spherules are surrounded by neutrophils (H&E).

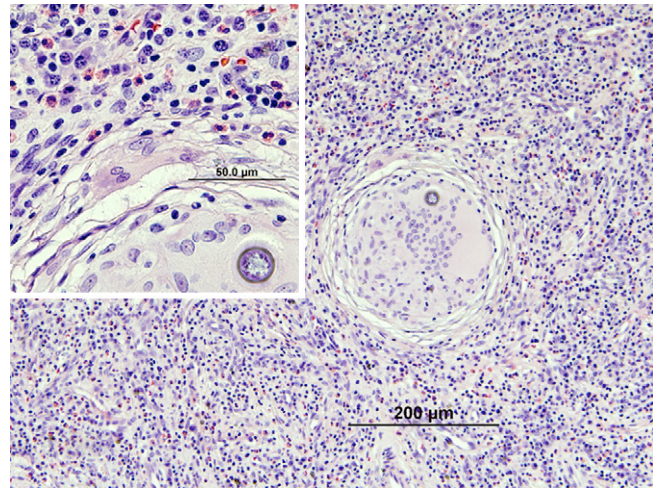


**Figure 5-27** Bronchoalveolar lavage sample from AIDS patient with disseminated coccidioidomycosis. (A) Mature spherule with endospores. (B) Non-sporulating spherules. Immature spherules seen to the left (Papanicolaou).

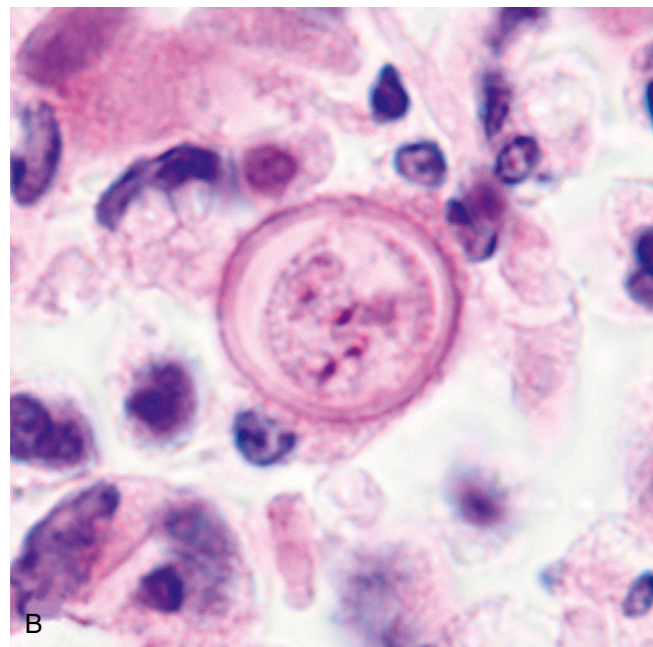
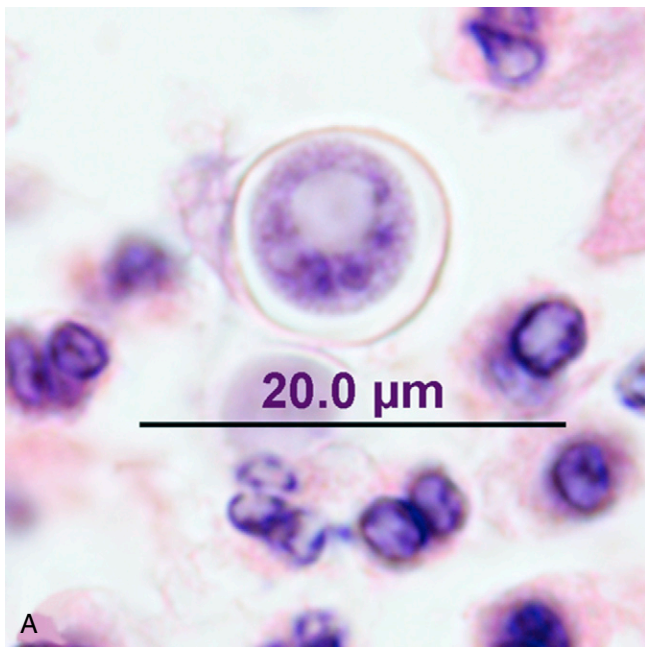




**Figure 5-28** Autopsy section of spleen from disseminated coccidioidomycosis in AIDS patient. Large, ruptured mature spherule containing endospores. Extensive necrosis, neutrophilic exudate and the presence of radiating stellate material indicative of Splendore–Hoeppli reaction (H&E).



**Figure 5-29** Lymph node biopsy from patient with chronic, disseminated coccidioidomycosis. Eosinophilic reaction surrounding a giant cell containing a spherule (H&E). Insert: an immature spherule within a giant cell. Infiltrate of eosinophils, lymphocytes and plasma cells in surrounding tissue (H&E, high power).



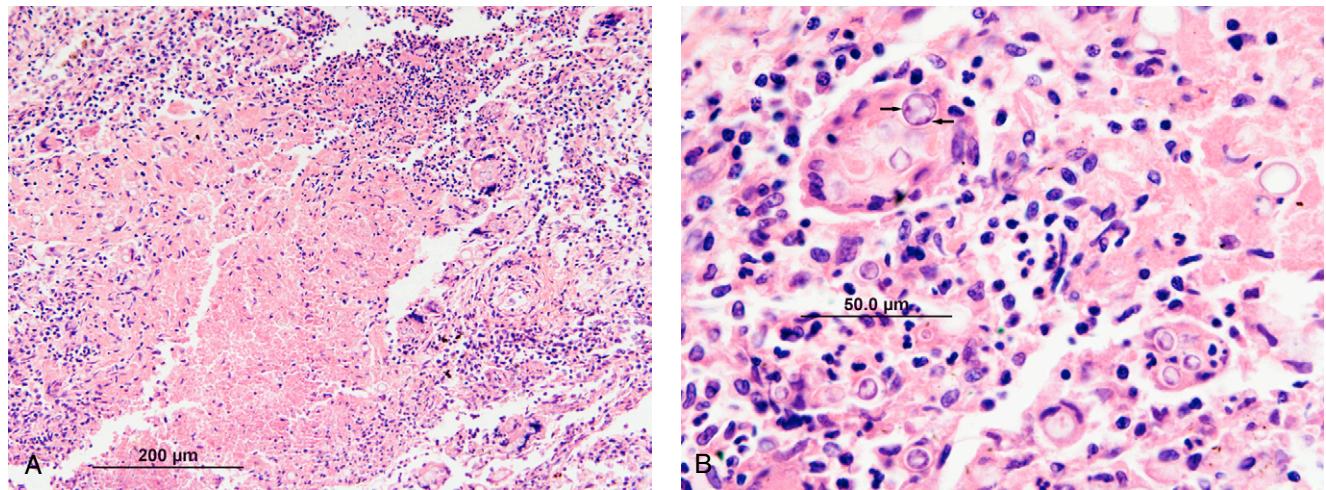
**Figure 5-30** Multinucleation in *B. dermatitidis*. (A) In H&E, nuclei are small, darkly stained dots (H&E). (B) Nuclei are somewhat accentuated by PAS-H stain (PAS-H).

#### *Paracoccidioides brasiliensis*

*Paracoccidioides brasiliensis* must be suspected in granulomatous or mixed granulomatous-suppurative disease of the lung or mucous membranes in patients from endemic parts of South and Central America. Although in the pre-AIDS literature, lymphoid tissue and gastrointestinal tract involvement is reported to be more pronounced in *P. brasiliensis* infections, this finding has been disputed.<sup>52</sup> Cutaneous and mucocutaneous lesions are typically secondary to primary pulmonary disease and accompanied by pseudoepitheliomatous hyperplasia.<sup>41</sup>

The only pathognomonic feature of *P. brasiliensis* is the presence of peripheral budding.<sup>41</sup> The latter may be very difficult to find and is best appreciated in GMS-stained slides. Differentiation of *Paracoccidioides* from *Coccidioides* and *Blastomyces* may be problematic (see Fig. 5-1). In the absence of the characteristic budding pattern, differentiation between *B. dermatitidis* and *P. brasiliensis* may not be possible. Important differences include the relatively narrow-necked budding and the more marked variation in size of *Paracoccidioides*. Immature spherules of *C. immitis* can resemble *P. brasiliensis*. Figure 5-31 illustrates pulmonary paracoccidioidomycosis with





**Figure 5-31** Pulmonary paracoccidioidomycosis. (A) Granuloma with central caseation surrounded by mononuclear cells and giant cells (H&E). (B) Large, spherical yeasts with thick cell walls primarily within giant cells. Multiple nuclei are present (arrows). A mixture of lymphocytes, macrophages and neutrophils are in background (H&E).



**Figure 5-32** Miniature forms of *P. brasiliensis* from laryngeal biopsy. Note presence of multiple peripheral blastoconidia (GMS).

caseating granulomata, regions of neutrophilic exudate and presence of large yeast forms with refractile, doubly contoured cell walls (Fig. 5-31B). Yeasts are mainly found within giant cells. *Paracoccidioides* yeasts vary from 4–60 µm in size, most being 30µm or less. As shown in Figure 5-32, a biopsy of laryngeal granulomata contained miniature cells of *P. brasiliensis* that are similar to *B. dermatitidis*.

### *Sporothrix schenckii*

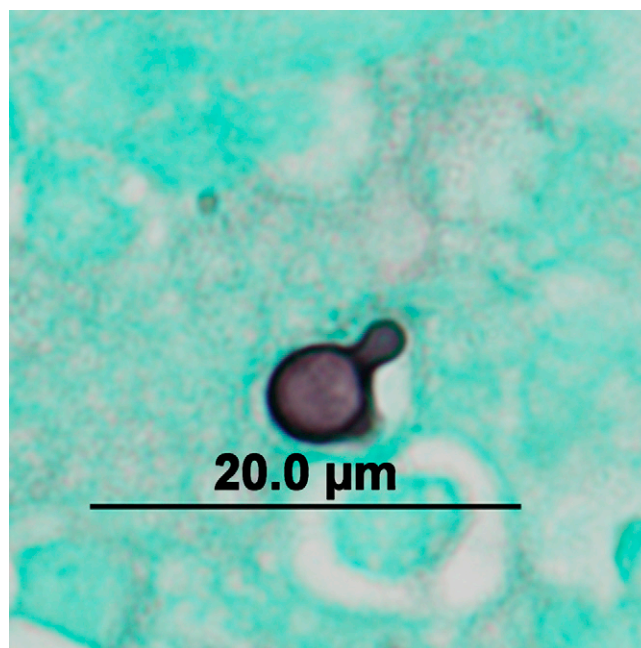
*Sporothrix schenckii* elicits a mixed granulomatous-suppurative inflammatory reaction. The usual portal of entry for *S. schenckii* is via lacerated or abraded skin.<sup>41</sup> Primary pulmonary sporotrichosis is less frequent, occurring mostly in men with alcoholism or chronic pulmonary disease.<sup>53</sup> Disseminated sporotrichosis complicating AIDS has been reported.<sup>54</sup>

Characteristic histologic findings of cutaneous lesions include prominent pseudoepitheliomatous hyperplasia. Yeasts are not usually recognized in H&E-stained sections and although best seen in GMS and PAS-stained slides, organisms may be difficult to find. A helpful but not pathognomonic finding is the so-called asteroid body which is an unfortunate term because

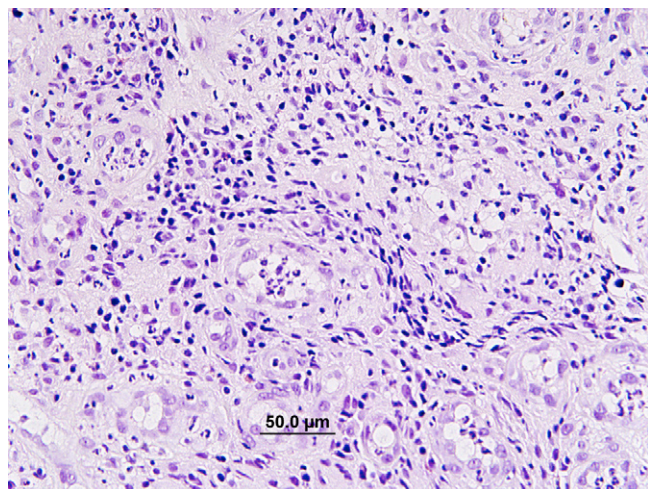
it implies a relationship to the non-infectious asteroid body found in sarcoid and some foreign body reactions. The “asteroid bodies” of sporotrichosis are stellate-shaped eosinophilic tissue reactions, forms of the Splendore–Hoepli phenomenon (HSP).<sup>41</sup> In sporotrichosis, the stellate-shaped HSP surrounds yeast cells. The latter can be identified by immunohistologic studies.<sup>55</sup> Sporotrichosis-associated HSP is more readily seen in H&E-stained slides than the yeast alone. Splendore–Hoepli reactions also can be seen around *Schistosoma* ova, bacteria and many other fungi such as *Cryptococcus*, *Candida*, and *Coccidioides* (see Fig. 5-28). In AIDS patients, granuloma formation may not be found, and abundant necrosis and variable numbers of neutrophils are seen.<sup>56</sup>

Primary pulmonary sporotrichosis may present as a solitary mass-like lesion, fibrocaceous granulomatous disease with cavitation, or in a miliary pattern.<sup>41</sup> *S. schenckii* is a globose, oval, or “cigar-shaped” yeast, 2–6 µm in diameter, but occasionally up to 10 µm (Fig. 5-33). Rarely, hyphae may be seen in the epidermis or in solid lesions from disseminated sporotrichosis.<sup>22</sup> Figure 5-34 illustrates a mixed granulomatous and suppurative inflammatory reaction in a case of pulmonary sporotrichosis.





**Figure 5-33** Lung biopsy from disseminated sporotrichosis. The yeast on the left has a long, cigar-shaped bud (GMS).



**Figure 5-34** Lung biopsy, same case as Fig. 5-33. A mixed granulomatous-neutrophilic inflammatory exudate (H&E).

## Mould infections

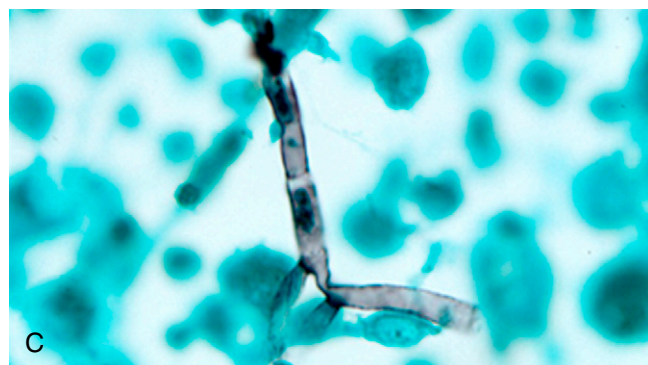
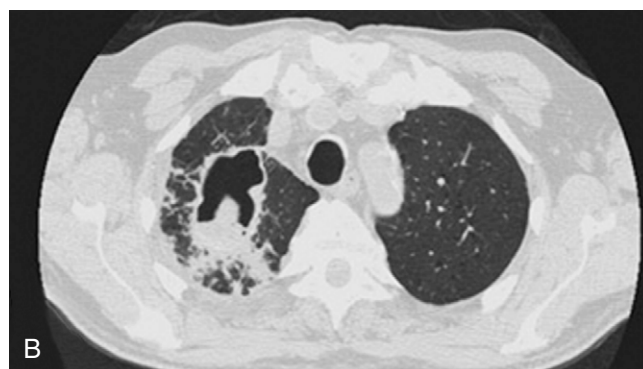
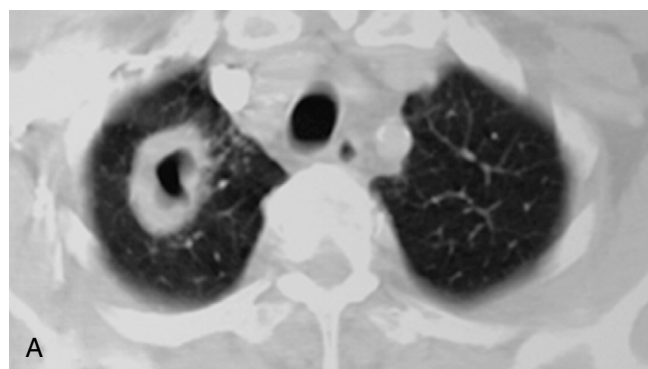
### *Aspergillus* spp.

Allergic *Aspergillus*-related disease is associated with asthma, atopy and cystic fibrosis.<sup>57-59</sup> *Aspergillus* colonization of nasal sinuses (see Fig. 5-4), bronchiectatic cavities or old tubercular or fungal cavities is relatively common. As shown in Figure 5-4, there is only mild chronic inflammation of the sinus wall, and neither invasion nor perifungal inflammation is seen. Figure 5-35 demonstrates the radiologic evolution of a fungus ball within a mycobacteria-induced cavity. Occasionally, fungal fruiting heads may be found in fungus balls colonizing preformed cavities, especially when the cavity is aerated.<sup>41</sup>

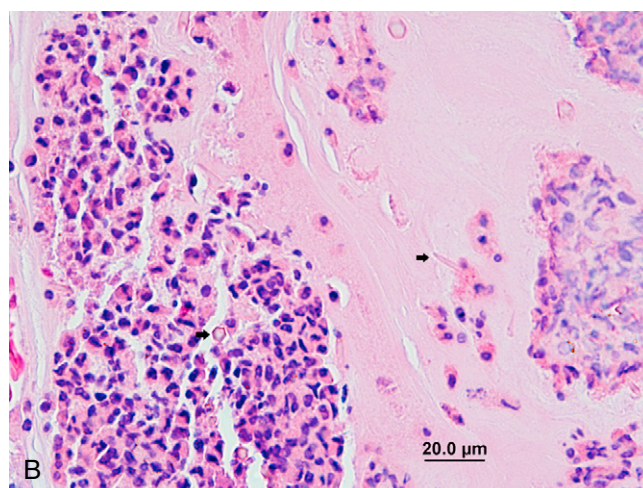
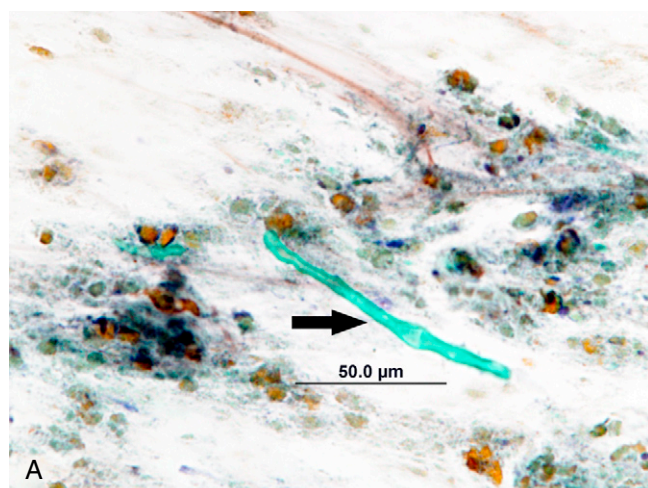
*Aspergillus* spp. and dematiaceous fungi are among the fungi most often associated with non-invasive, allergic disease. The hallmark of this entity is AM, found predominantly in the lungs and one or more paranasal sinuses.<sup>17,57</sup> Evacuation of contents of involved sinuses typically yields grumous material that is foul-smelling and has the consistency of “peanut butter.”<sup>60</sup> In cytologic preparations of AM, the background may appear granular and amorphous, suggesting the possibility of necrotizing neoplasia; however, the presence of mucin, degenerating eosinophils and Charcot-Leyden crystals allows one to make the correct diagnosis<sup>61</sup> (Fig. 5-36). Fungal elements may be difficult to find in both histologic and cytologic preparations.

Bronchial mucoid impaction with presence of AM and fungi, but lacking the other criteria for allergic bronchopulmonary aspergillosis (ABPA), may also occur. Although infrequently biopsied, the histopathology of ABPA has been described in a few publications.<sup>59,62-64</sup> Mucoid impaction and/or bronchocentric granulomatosis are typically found. In the former, proximal bronchi are dilated, obstructed and filled with laminated-appearing AM that often contains hyphae. In bronchocentric granulomatosis, there is an abrupt transition from the uninvolved bronchiole to a region in which the bronchiolar wall has been replaced by granulomatous inflammation composed of epithelioid macrophages. Degenerating neutrophils, eosinophils, and necrotic debris may be seen in bronchiolar lumina distal to granulomatous bronchiolitis, and often there is a peribronchial or bronchiolar infiltrate of lymphocytes, plasma cells and eosinophils.<sup>65</sup> Pulmonary fibrosis may occur in later stages.<sup>59</sup>

Subacute pulmonary invasive aspergillosis<sup>66-68</sup> differs from the aggressive, vasoinvasive type. Denning et al have recently proposed the term subacute invasive pulmonary aspergillosis for these more indolent forms of invasive aspergillosis.<sup>66</sup> Aspergillosis is not common in AIDS patients, and interestingly,



**Figure 5-35** Fungus ball in mycobacteria-induced lung cavity. (A) Radiograph showing a thick-walled cavity. (B) Radiograph performed 1 year later showed a thin-walled cavity with a dependent mass. (C) Fine needle aspiration contained hyphae morphologically consistent with *Aspergillus* spp. (GMS).



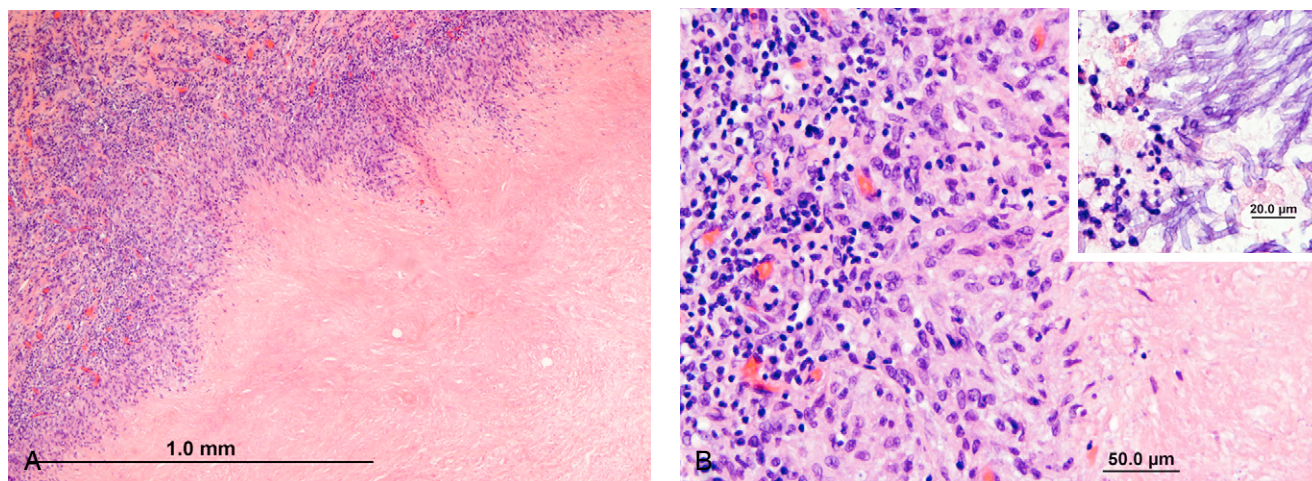
**Figure 5-36** Allergic fungal sinusitis, allergic mucin. (A) Cytologic preparation of paranasal sinus showing presence of strands of mucin, degenerating eosinophils and hypha (arrow) (Papanicolaou). (B) Histologic section from surgical evacuation of sinuses. Abundant mucin, eosinophils and Charcot-Leyden crystals (arrows) (H&E).

aspergillosis in these patients often is manifest as subacute invasive disease, with circumscribed abscesses surrounded by fibrosis, rather than the aggressive invasive and disseminated disease that is usually associated with severe neutropenia.<sup>69</sup> Figure 5-37 illustrates the histology of a right upper lobe resection.

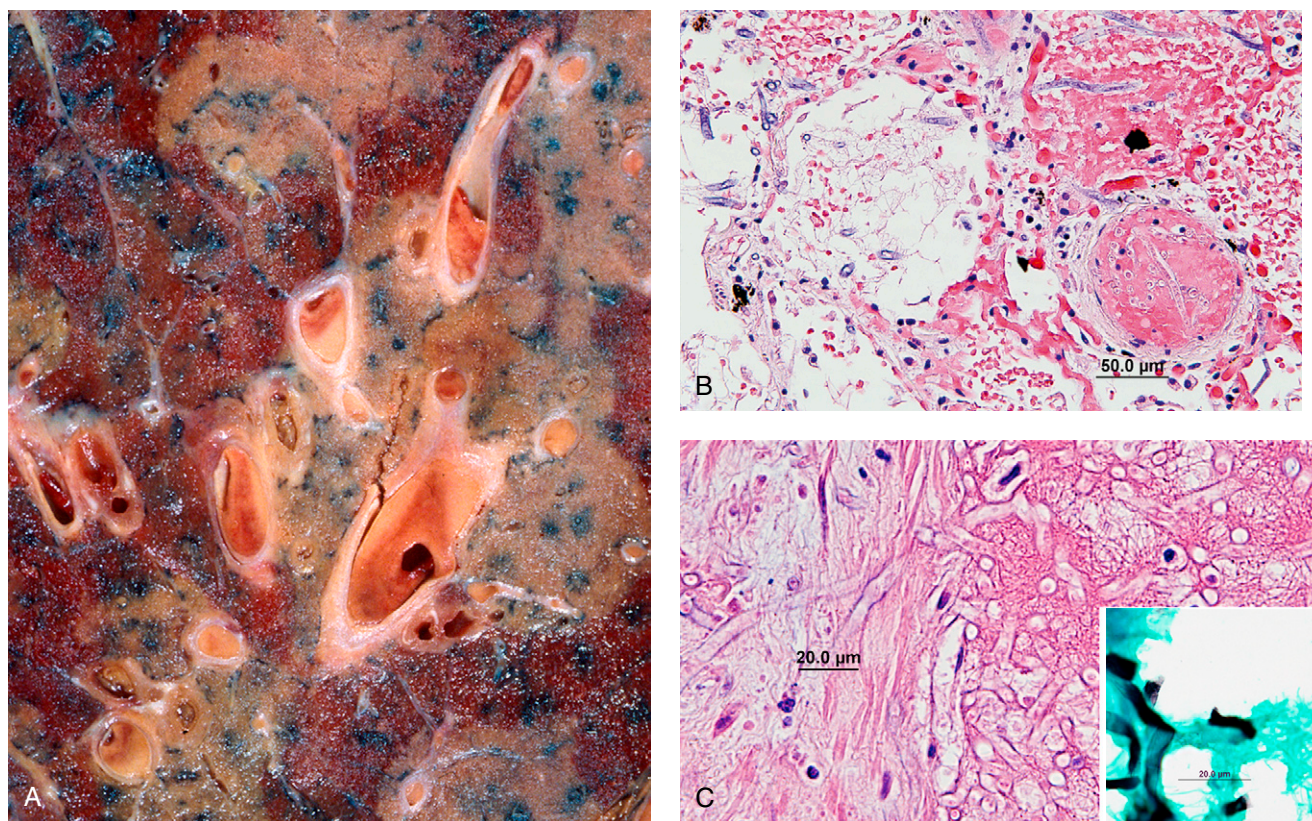
In the aggressive, invasive form of pulmonary aspergillosis, widespread blood vessel invasion, thrombotic vascular occlusions, hemorrhagic and coagulative necrosis are characteristic, and extrapulmonary hematogenous dissemination is not uncommon. Because neutropenia is usually present in aggressive invasive aspergillosis, coagulative

necrosis and hemorrhage are the dominant histologic findings. Neutrophils and their accompanying liquefactive necrosis are less frequently seen. Multiple, nodular infarcts, either pale or hemorrhagic, and laminated, intravascular thrombi are seen in lung (Fig. 5-38A). In histologic sections, large numbers of hyphae fill blood vessel lumina, invade vascular walls and extend through necrotic lung parenchyma (see Fig. 5-6B; Figs 5-38B, C). Hyphae within the lumina of blood vessels often have a stellate coating of plasma and fibrin, giving them a spiculated appearance (Fig. 5-38C). Depending upon the degree of vascular perfusion of the affected lung, one may see regions of





**Figure 5-37** Cavitory lung lesion. (A) Circumscribed granulomatous nodule with central necrosis surrounded by a mononuclear cell infiltrate (H&E). (B) Wall of lesion contains epithelioid macrophages surrounded by an infiltrate of lymphocytes and plasma cells. Insert: central portion of the necrotizing lesion contains fungi morphologically consistent with *Aspergillus* sp. (H&E).



**Figure 5-38** Invasive aspergillosis in an AIDS patient who had severe neutropenia. *A. glaucus* was cultured. (A) Gross section of lung with pale, rounded areas of infarction surrounding thrombosed pulmonary arteries. (B) Histologic section showing intravascular and extravascular hyphae, extravasated erythrocytes and fibrin (H&E). (C) Section of blood vessel. Intravascular hyphae are surrounded by a stellate rim of fibrin (H&E). Insert: touch preparation of one of the intravascular thrombi seen in (A). Hyphae and fibrin present (GMS).



coagulative necrosis (see Fig. 5-6B) or hemorrhagic necrosis (see Fig. 5-38B). Owing to hematogenous dissemination, fungal infarcts may be found within other organs, such as brain, intestinal tract, heart, kidneys and spleen. Ulcerative *Aspergillus* tracheobronchitis has also been described.<sup>70</sup>

Typical hyphae of *Aspergillus* have parallel walls, measure 3–6  $\mu\text{m}$  in diameter, are septate, and have dichotomous branching (Fig. 5-39A). The morphologic features of these hyphae, however, are not specific for *Aspergillus* spp.; other fungi, particularly *Pseudallescheria boydii* and *Fusarium* spp., cannot be definitively distinguished by morphologic characteristics alone. Occasionally, especially when the fungal hyphae are exposed to air, fruiting heads may be found in tissue, allowing identification of *Aspergillus* spp. (Fig. 5-39B). Oxalate crystals may be seen in histologic sections from patients with *A. niger*. Fatal pulmonary oxalosis secondary to *A. niger* infection has been reported.<sup>71,72</sup>

## Zygomycetes

The zygomycetes have a propensity to invade blood vessels, frequently causing arterial or venous thromboses and subsequent ischemic or hemorrhagic infarction<sup>73–75</sup> (Fig. 5-40). Invasive pulmonary zygomycosis, which histologically resembles invasive pulmonary aspergillosis, is commonly associated with hematopoietic malignancies, particularly patients with neutropenia.<sup>75–77</sup> Embolization of intravascular hyphae may result in widespread dissemination. Zygomycetes typically elicit a neutrophilic reaction, although in granulocytopenic persons inflammation may be minimal. Aggregates of epithelioid histiocytes may be seen peripheral to regions of acute, necrotizing inflammation in more long-standing infections (Fig. 5-40C).

A lesser known form of zygomycosis, endobronchial zygomycosis, is a chronic, locally invasive disease similar to subacute pulmonary aspergillosis.<sup>78</sup> Fungus balls are found predominantly

within a bronchial lumen, and there may be chronic necrotizing invasion of the bronchial wall resulting in cavitation, bronchiectasis and fibrosis. Although this is a relatively indolent form of zygomycosis, severe hemorrhage from erosion into hilar blood vessels is a lethal complication of endobronchial zygomycosis.

Zygomycete hyphae are easily visualized in H&E and Papanicolaou stains (see Figs 5-2B, 5-40; Fig. 5-41), but often stain weakly by the GMS method. Characteristic hyphae are broad, thin-walled, pleomorphic, irregular branching, 5–20  $\mu\text{m}$  in diameter, and frequently twisted, folded, wrinkled or collapsed (see Fig. 5-41). Although the hyphae typically are described as non-septate, they are actually sparsely septate. Rarely, in lesions exposed to ambient air, round to oval, thick-walled chlamydoconidia, 15–30  $\mu\text{m}$  diameter, may be formed<sup>79</sup> (Fig. 5-42).

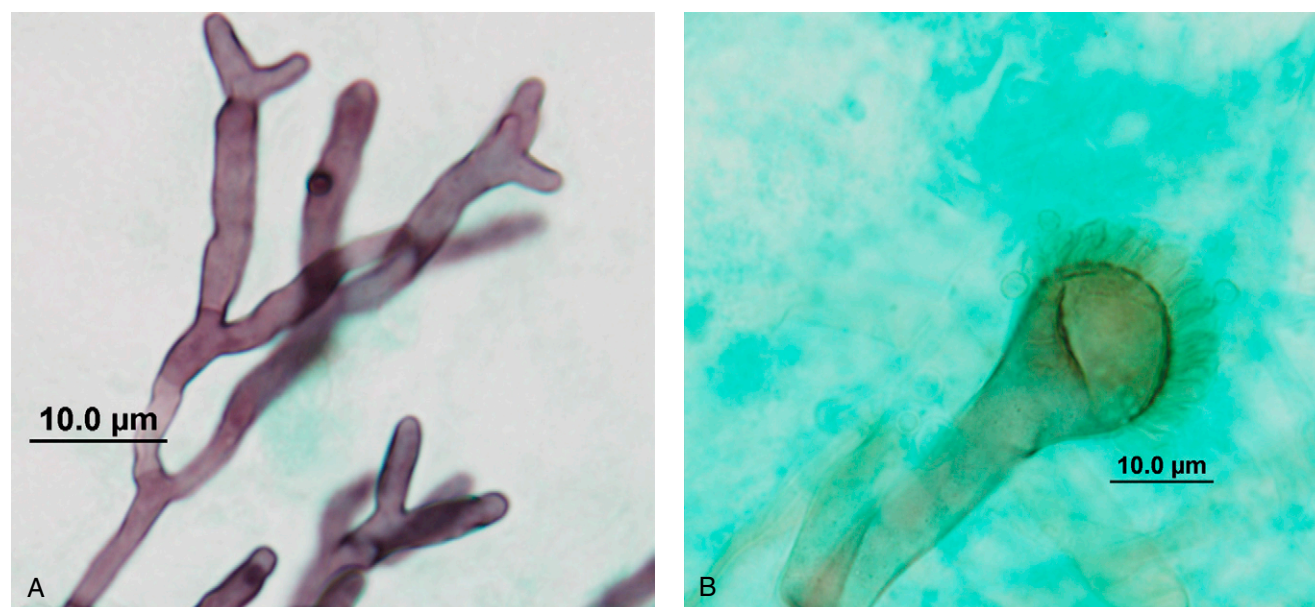
## Less common hyaline mould infections

### *Fusarium* spp.

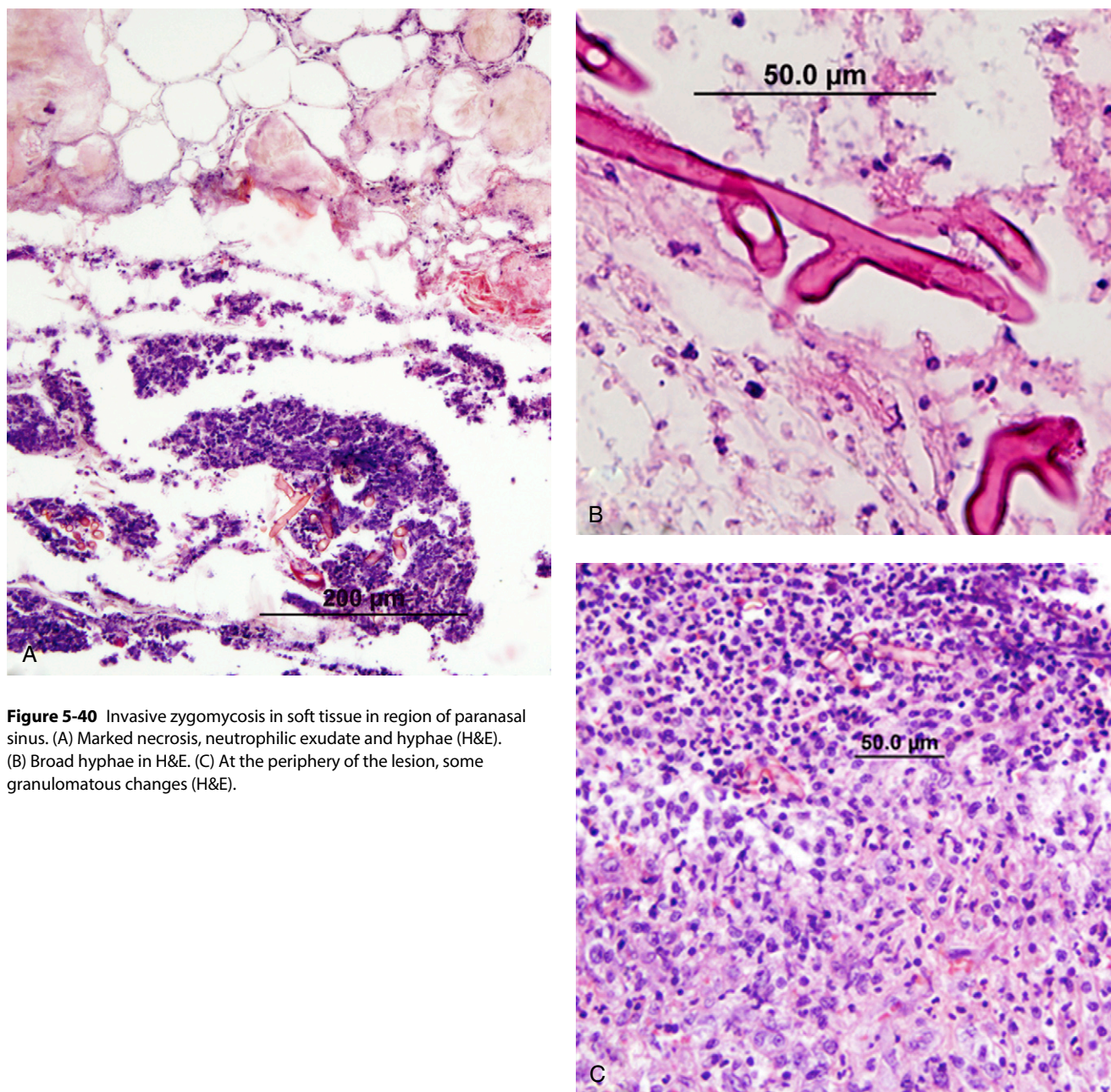
In severely burned and immunosuppressed patients, dissemination may occur.<sup>80</sup> The histopathology of fusariosis is virtually identical to that of the aggressive form of invasive aspergillosis.<sup>81</sup> Blood vessel invasion and infarcts with accompanying coagulative necrosis and hemorrhage are common. If neutrophils are present, liquefactive necrosis and abscess formation may be seen (Fig. 5-43). Hyphae of *Fusarium* spp. in tissue measure 3–8  $\mu\text{m}$  in diameter, have septa, and are similar to *Aspergillus*. A useful, but not pathognomonic clue to the identity of *Fusarium* spp. is the presence of constrictions at the site of septa, hyphal varicosities, and terminal or intercalated vesicles (see Figs 5-2C, 5-43).

### *Pseudallescheria boydii*

*Pseudallescheria boydii* is commonly associated with mycetomata in the United States.<sup>22,41</sup> It also may produce a fungus ball resembling a pulmonary aspergilloma, and invasive



**Figure 5-39** *Aspergillus fumigatus* in bronchial brushing from a cancer patient with tracheobronchitis. (A) Hyphae. (B) Fruiting body with conidiation typical of *A. fumigatus* (GMS).



**Figure 5-40** Invasive zygomycosis in soft tissue in region of paranasal sinus. (A) Marked necrosis, neutrophilic exudate and hyphae (H&E). (B) Broad hyphae in H&E. (C) At the periphery of the lesion, some granulomatous changes (H&E).

pseudallescheriasis mimics invasive aspergillosis,<sup>82</sup> with nodular infarcts secondary to angioinvasion by the fungus and necrotizing pneumonitis with abscesses in non-granulocytopenic hosts. In tissue, the septate hyphae of *P. boydii* are difficult to distinguish from those of aspergilli, although they are somewhat narrower, measuring 2–5 µm in width, and their pattern of branching is more random.

## Dematiaceous fungal infections

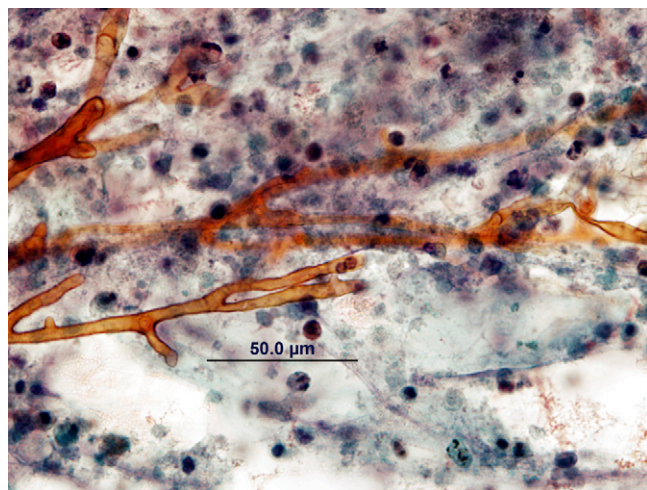
### Chromoblastomycosis

Chromoblastomycosis describes a cutaneous infection caused by a variety of dematiaceous fungi.<sup>83</sup> The portal of entry is typically via direct traumatic inoculation of the skin. Detailed

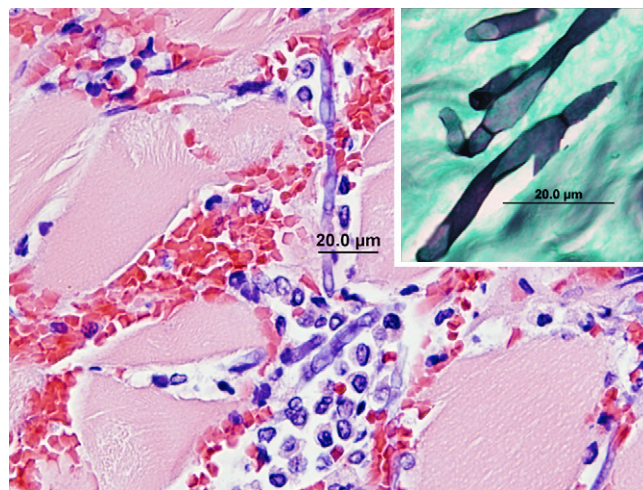
descriptions of the gross and histopathologic aspects of this disease were reported by Carrión.<sup>84,85</sup> Most cases are not associated with immunosuppressive diseases.

Chromoblastomycosis is characterized by marked hyperkeratosis, parakeratosis and pseudoepitheliomatous hyperplasia that, like cutaneous blastomycosis, paracoccidioidomycosis and coccidioidomycosis, may be misdiagnosed as squamous cell carcinoma (Fig. 5-44A). Within the dermis, one sees epithelioid and giant cell histiocyte proliferation with foci of neutrophil-rich microabscesses (Fig. 5-44B). Fungal elements are most commonly found within dermal macrophages and mainly consist of sclerotic bodies or muriform cells 5–12 µm in diameter (Fig. 5-44B insert). Occasionally, pigmented hyphae or moniliform hyphae are present. Agents of chromoblastomycosis are

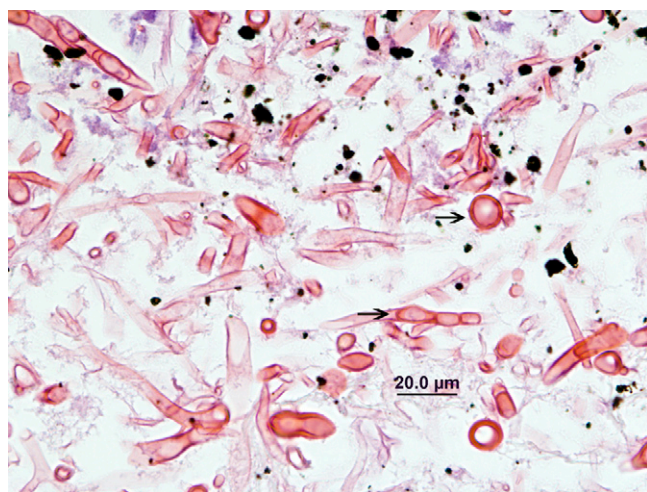




**Figure 5-41** Fine needle aspirate of a zygomycotic renal abscess (Papanicolaou).



**Figure 5-43** Skeletal muscle from burn patient with invasive fusariosis. Hyphae with intercalated mycelial swellings. There is a neutrophilic inflammatory reaction and hemorrhage (H&E). Insert: constrictions are seen in the region of the septa (GMS).



**Figure 5-42** Endobronchial zygomycosis with formation of thick-walled vesicles (arrows) terminally and intercalary (H&E).

dark brown to golden, although in some cases the pigmentation is appreciated only by a melanin stain. These morphologic features, however, do not distinguish the different fungi that can cause chromoblastomycosis.<sup>83</sup>

#### Phaeohyphomycosis

Phaeohyphomycosis, especially the systemic form, is usually associated with immunosuppression. In phaeohyphomycosis, hyphae and moniliform hyphae and pseudohyphae predominate.<sup>83,86</sup> Subcutaneous cyst-like lesions, so-called phaeomycotic cysts, are characterized by central necrosis, fibrin and a neutrophilic infiltrate surrounded by epithelioid histiocytes and fibrosis. Aspiration yields a mixture of epithelioid histiocytes,

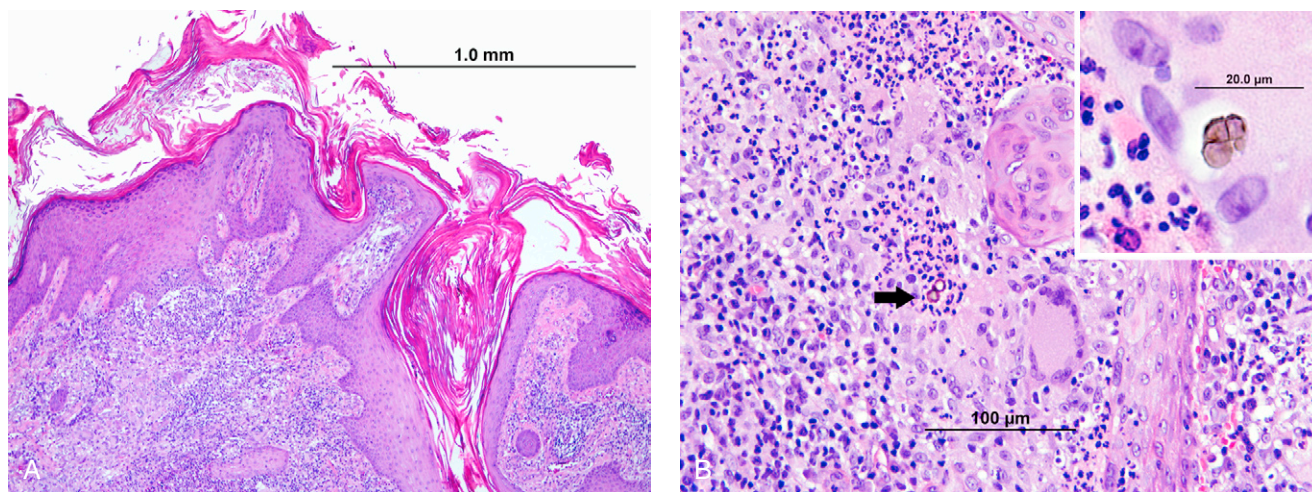
giant cells and fungi (Fig. 5-45). Early lesions may consist of stellate abscesses rather than a cyst. Subcutaneous phaeohyphomycosis may present as non-necrotizing, subcutaneous granulomata (Fig. 5-46). Visceral phaeohyphomycosis has a mixed, granulomatous and suppurative inflammatory reaction, and the appearance of the organisms in tissue is similar to that of subcutaneous phaeohyphomycosis (see Fig. 5-2D). Hyphae are seen within the center of the lesion, appearing hyaline to golden brown on both H&E-stained sections and Papanicolaou-stained cytology preparations. Typical hyphae are 2–6 µm wide and of variable length. They are septate, sometimes branched, and occasionally contain thick-walled vesicular swellings, up to 25 µm in diameter (see Figs 5-2D, 5-45, 5-46). The brown pigmentation in the fungal cell walls usually is apparent in H&E-stained tissue sections. Stains for melanin will reveal the dematiaceous nature of the fungi when it is not obvious.

#### Miscellaneous fungi associated with eumycetoma

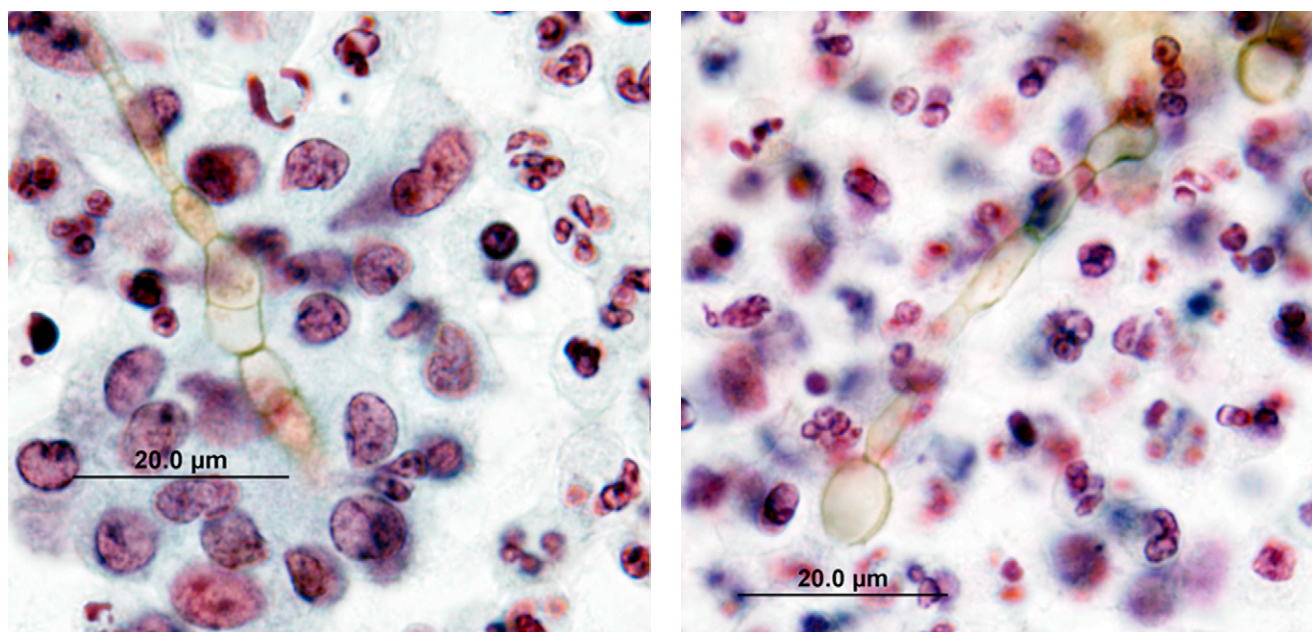
Mycetomata are characterized by a tumor-like presentation, draining sinuses, and grains or granule-like structures composed of masses of organized microbes surrounded by inflammatory cells.<sup>41</sup> These lesions may be caused by actinomycetes, other types of bacteria (botryomycosis), or fungi (eumycotic mycetomata).<sup>87,88</sup> The color and texture of the grains vary with causative etiologic agent.<sup>22,87,88</sup>

In histologic preparations, the granule of eumycotic mycetomata consists of a tangle of hyphae admixed with amorphous material (Fig. 5-47). Surrounding the organisms is a rim of neutrophils. A Splendore–Hoepli reaction may be seen between the fungi and the inflammatory cell reaction. Macrophages and fibrosis typically surround older lesions. The size of the hyphae varies, and vesicles may be seen, particularly near the periphery of the granule. The fungal hyphal morphology can assist with the identification of causative agent in some instances.





**Figure 5-44** Chromoblastomycosis, skin biopsy. (A) Hyperkeratosis and pseudoepitheliomatous hyperplasia (H&E). (B) Within the dermis, mixed inflammatory infiltrate composed of epithelioid macrophages and giant cells, lymphocytes, plasma cells and foci of neutrophils. Brown-colored sclerotic cells (arrow) (H&E). Insert: a multiseptate sclerotic body within a giant cell (H&E).



**Figure 5-45** Fine needle aspiration of a subcutaneous, fluctuant mass in the ankle that had been clinically diagnosed as a varicose vein. *Phialophora verrucosa* was cultured. Aspirate contains a mixture of macrophages and neutrophils (Papanicolaou).

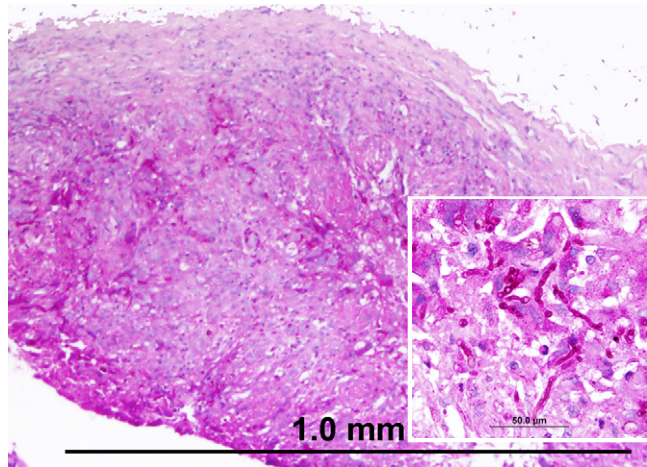
## Less common fungal infections

### *Trichosporon* spp.

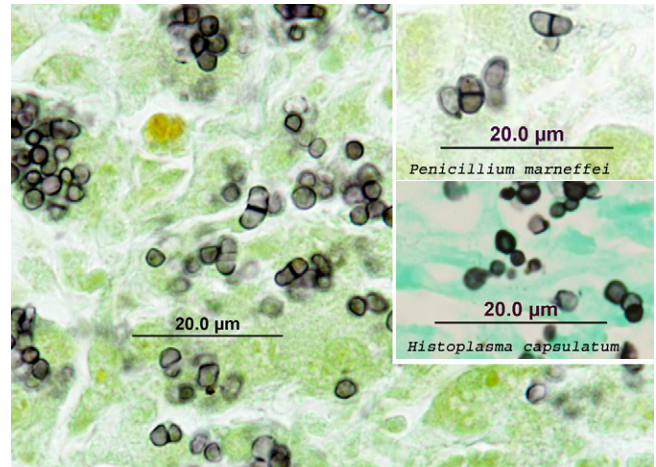
*Trichosporon beigelii* can cause disseminated infection in immunocompromised patients.<sup>89</sup> Parenchymal lesions of disseminated trichosporonosis are a result of vascular invasion by the fungus and subsequent hematogenous spread. The lesions resemble those of invasive, systemic candidiasis or aspergillosis

(i.e., necrotic nodules composed of fungal elements proliferating with a radial pattern of growth). Abscesses or granulomatous lesions may occur. In tissue, *T. beigelii* produces pleomorphic yeast cells, measuring 3–8 µm in diameter, septate hyphae, and arthroconidia, either of which can predominate. The organism can readily be confused with *Candida* spp.<sup>90</sup> The presence of arthroconidia, if observed, distinguishes *Trichosporon* from *Candida* species.

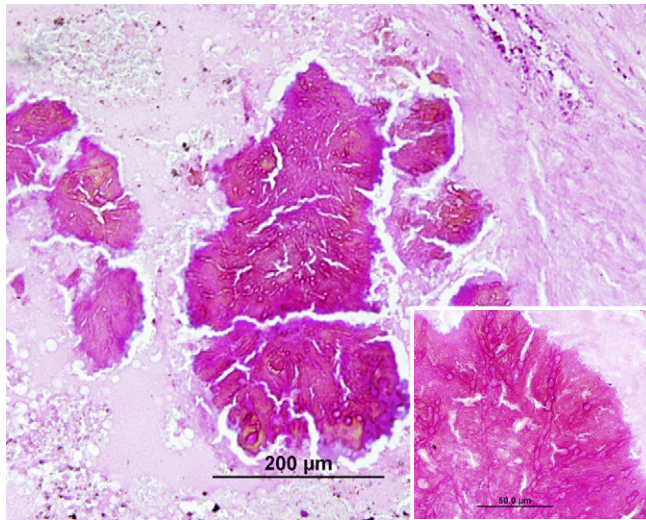




**Figure 5-46** Subcutaneous phaeohyphomycosis, biopsy of skin nodule. Subcutaneous granuloma composed largely of epithelioid macrophages. (PAS-H). Insert: Moniliform hyphae. Pigmentation is faint (PAS-H).



**Figure 5-48** *Penicillium marneffei* compared to *H. capsulatum*: *P. marneffei* is round to allantoid compared to the oval *H. capsulatum*, and reproduces by fission rather than budding (GMS).



**Figure 5-47** Eumycotic mycetoma. A deeply eosinophilic granule is surrounded by amorphous material and fibrosis (PAS-H). Insert: hyphae are admixed amorphous material (PAS-H).

### *Penicillium marneffei*

*Penicillium marneffei* is a dimorphic fungus whose endemicity is limited to South Eastern and Far Eastern Asia. The majority of infections have been reported in immunosuppressed patients infected with HIV.<sup>91</sup> The clinical presentation and histopathologic findings in *P. marneffei* infections are very similar to those of *H. capsulatum*.<sup>92</sup> Prior to the AIDS epidemic, *P. marneffei* infections were infrequent.<sup>54</sup> Like *H. capsulatum*, defense against *P. marneffei* is largely mediated by macrophages, and the fungus occurs predominantly within cells of the mononuclear phagocyte system. Mootsikapun and Srikulbutr found both the clinical and histopathologic similarities between disseminated histoplasmosis and penicilliosis to be striking, and differentiation between the two diseases to be difficult.<sup>91</sup> Cells of *P. marneffei* do not bud, but divide by fission with a single transverse septum (Fig. 5-48).

## Infections caused by fungi of uncertain classification

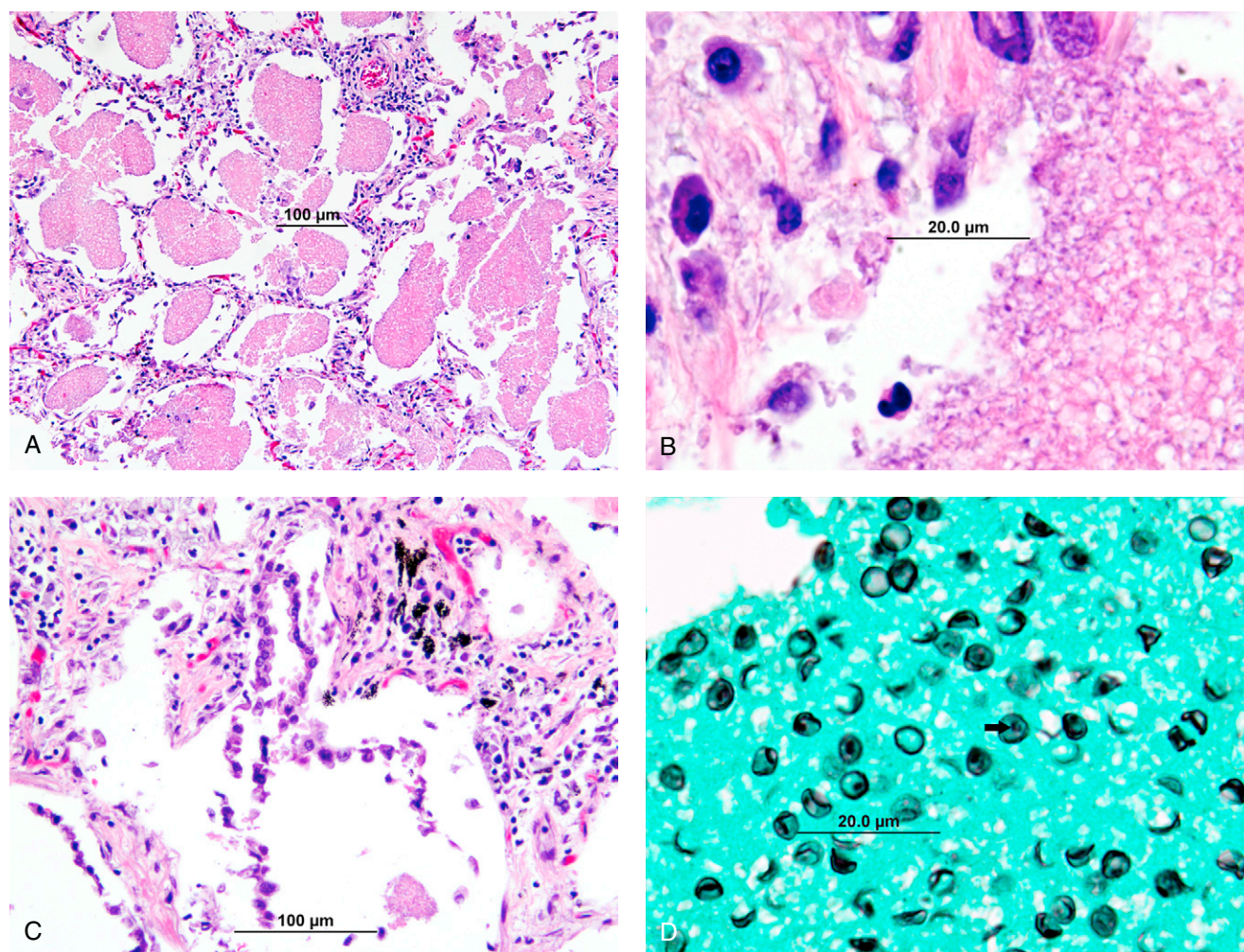
### *Pneumocystis jiroveci*

*Pneumocystis jiroveci* (formerly *P. carinii*) classically was reported to be associated with plasma cell interstitial pneumonia in malnourished European children during and following World War II.<sup>93</sup> Today, *P. jiroveci* pneumonia (PJP) is a disease of the T cell immunosuppressed typically occurring in transplant recipients, and is the most common opportunistic infection in AIDS patients.<sup>94</sup> In histologic sections, the alveolar walls and pulmonary interstitium are thickened and contain a lymphoplasmacytic infiltrate. The organisms are predominantly found within the alveolar spaces as a “foamy exudate.” The latter is actually a conglomerate of *P. jiroveci* cysts, extracystic bodies and protein-rich fluid.<sup>94</sup>

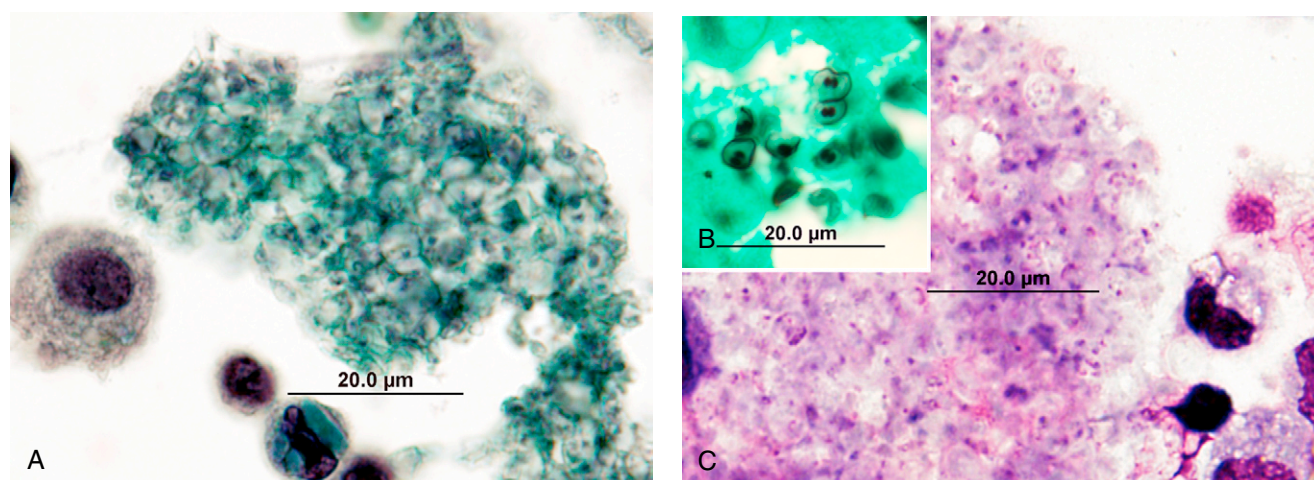
The morphologic features of PJP are seen in Figures 5-49 and 5-50. Aggregates of organisms are typically observed within the alveolar spaces, and frequently there is a clear zone between the alveolar wall and the mass of fungi (see Fig. 5-49A). *P. jiroveci* are seen as so-called “foamy exudates,” which under high power magnification consist of soap bubble-like material containing tiny dark-staining dots (see Figs 5-49A,B, 5-50A). To properly evaluate a GMS-stained slide for *Pneumocystis* (see Fig. 5-49D), it is important that the silver solution is not allowed to precipitate for a prolonged period of time because oversteining obliterates important diagnostic features. In a properly stained section or cytologic preparation, *Pneumocystis* cysts appear as round structures, 5–6µm in diameter. There is a characteristic thickening within the cyst wall that is seen as a black dot or, in very well-stained preparations, a pair of closely apposed dots (see Figs 5-49D, 5-50B). Within some cysts, developing intracystic forms may be seen as either a solid mass of blue and purple protoplasm or up to eight individual intracystic bodies (see Figs 5-21B, 5-50C).

The so-called atypical reactions to PJP include diffuse alveolar damage, granulomata, pneumocystoma and intrapulmonary and subpleural cyst formation.<sup>95,96</sup> Diffuse alveolar damage



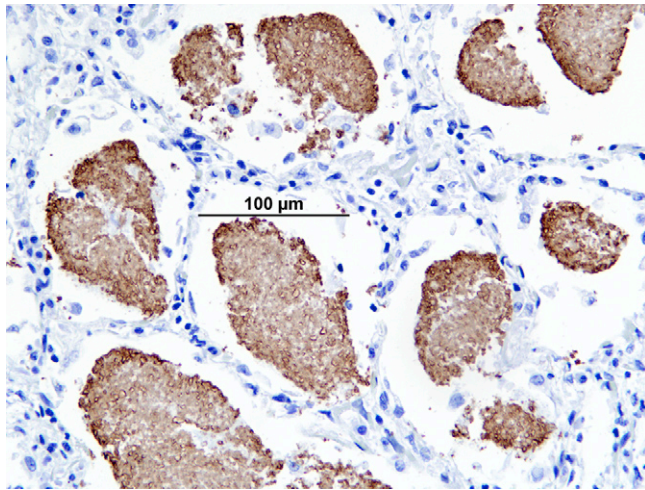


**Figure 5-49** Pulmonary pneumocystosis, lung section. (A) Eosinophilic “exudate” fills the alveolar spaces with a clear space between the “exudate” and the alveolar walls (H&E). (B) Plasmacytic infiltrate in alveolar wall (left) and bubbly exudate (right) containing tiny dark dots (H&E). (C) PJP with evidence of alveolar damage. There is interstitial thickening and marked type II pneumocyte hyperplasia (H&E). (D) *Pneumocystis* cysts within an alveolar space. There are the typical darkly stained dots (arrow) indicative of thickenings in cyst wall (GMS).

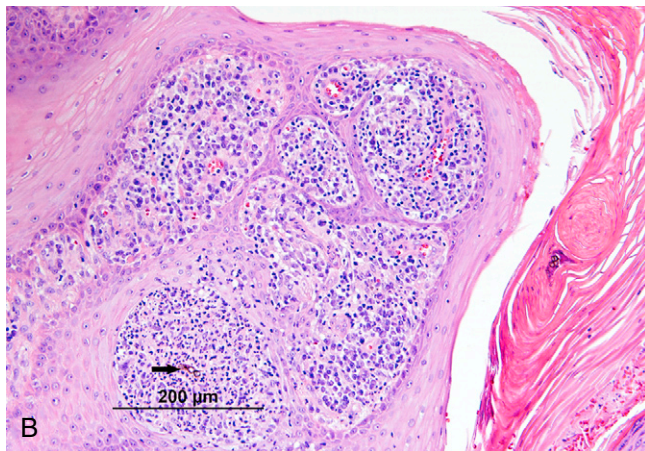
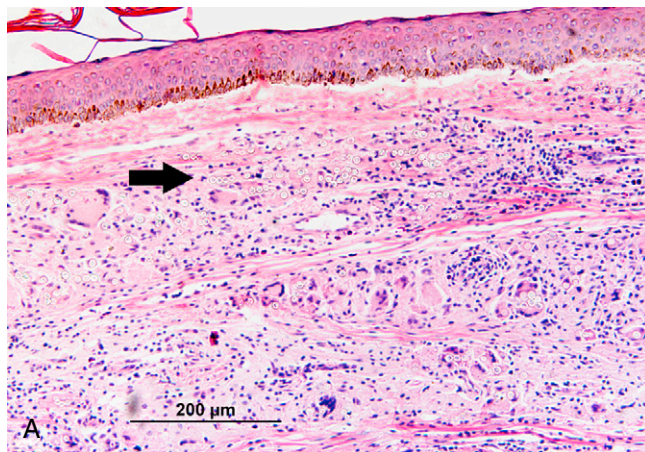


**Figure 5-50** *P. jiroveci* in bronchoalveolar lavage. (A) *Pneumocystis* appears as bubbly exudate containing central, dark-staining dots (Papanicolaou). (B) Well-stained cysts containing two parallel dots within the cyst wall (GMS). (C) In Romanowski-stained preparations, “exudate” consists of clear appearing cysts containing intracystic bodies and extracystic trophic forms with purple-staining nuclei and bluish cytoplasm (Giemsa).





**Figure 5-51** Immunostain of *P. jiroveci* in lung section. Cysts and extracystic forms (Novocastra Laboratories, immunostain).



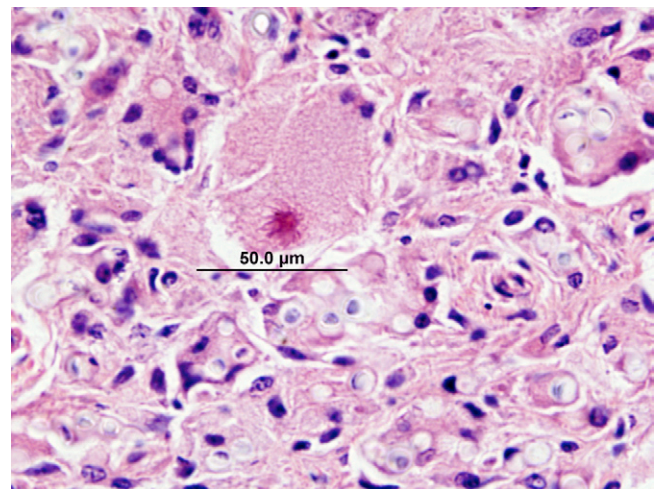
**Figure 5-52** (A) Lacaziosis. Atrophic epithelium containing a relatively normal “grenz zone” just beneath the epidermis. Fungi are refractile, clear spheres (arrow) within an inflammatory reaction (H&E). (B) Chromoblastomycosis. Marked pseudoepitheliomatous hyperplasia and hyperkeratosis. Two small, brown sclerotic cells (arrow) are seen within a dense dermal inflammatory reaction (H&E).

(DAD), or organizing interstitial pneumonia, often accompanies PJP, especially in AIDS patients who may clinically present with acute respiratory distress syndrome (ARDS). Neutrophilic response and abnormalities in surfactant likely contribute to the evolution of DAD.<sup>94</sup> In cases of DAD, intraalveolar neutrophils, hyaline membranes, type II pneumocyte hyperplasia and interstitial, intraalveolar and intrabronchiolar fibrosis may be seen, depending upon the stage of the disease. Granulomata with caseation may occur, and some have related this phenomenon to therapy with inhaled pentamidine.<sup>96</sup> Both immunohistochemical and fluorescent antibody tests are commercially available for detection of *P. jiroveci* (Fig. 5-51).<sup>97,98</sup>

### Lacazia loboi

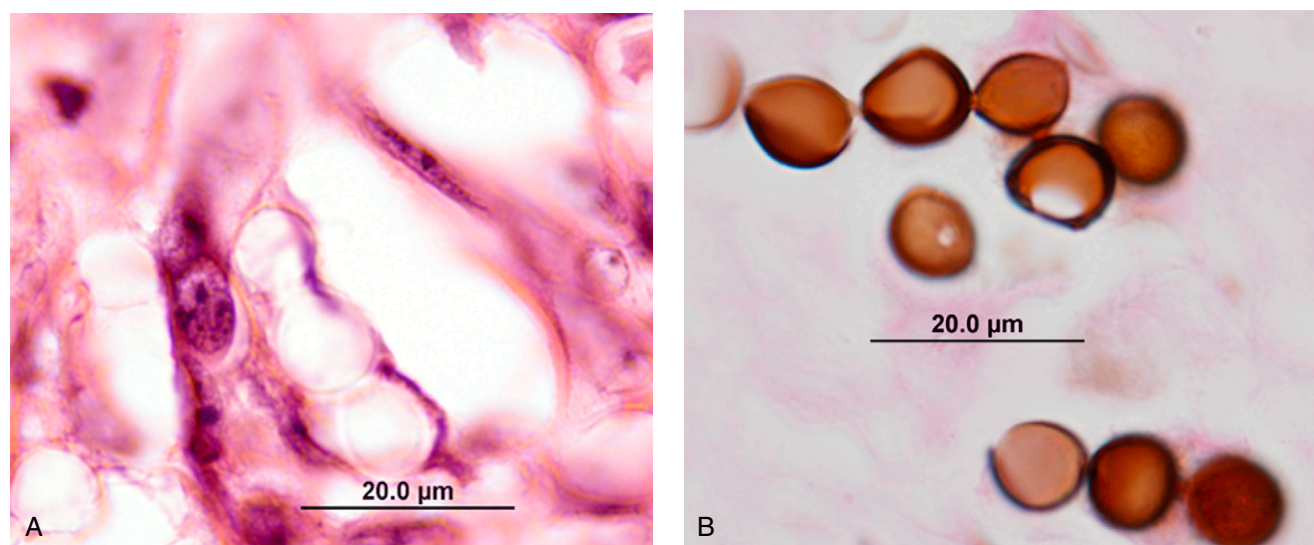
The overlying skin often appears shiny and atrophic, although older lesions may be verrucoid.<sup>41,54</sup> The epidermis is usually atrophic. Pseudoepitheliomatous hyperplasia and hyperkeratosis are not usually seen. Figure 5-52 shows the comparative histopathology of lacaziosis (syn. lobomycosis) and chromoblastomycosis. In older lesions there may be some hyperkeratosis and ulceration. Pathologic alterations of lacaziosis are limited to the subcutaneous tissue where there are sheets of epithelioid macrophages and many giant cells. Common findings in lacaziosis are asteroid bodies within the cytoplasm of giant cells (Fig. 5-53). These are true asteroid bodies that appear similar to those found in sarcoid and some other foreign body reactions.<sup>55</sup> Fungal organisms are not present within the asteroid body, in contrast to the Splendore-Hoeppli phenomenon that may accompany sporotrichosis and other fungal infections.

The fungi appear as clear, round structures with thick, refractile cell walls (see Fig. 5-52), often within epithelioid macrophages and giant cells. Yeast is relatively uniform in size and shape, approximately 10 μm in diameter. They vary far less in size and shape than *Paracoccidioides*, a fungus found in the same geographic regions as *L. loboi*. *L. loboi* is often arranged in short chains connected by a tube-like structure (Fig. 5-54).



**Figure 5-53** Lacaziosis in skin biopsy with asteroid body. A typical asteroid body within a giant cell. Yeast cells are within surrounding macrophages (H&E).





**Figure 5-54** Lacaziosis in skin biopsy. (A) Fungi with clear, refractile, thick cell walls are arranged in a short chain (H&E). (B) A short tube-like structure connects the yeast cells (GMS).

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# Radiology of fungal infections

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Radiology plays an important role in the diagnosis of fungal infections although the radiologic manifestations are usually non-specific. It is typically the clinical status of a patient more than the radiologic findings that will raise the index of suspicion. The radiologic investigation of patients with mycotic infections is usually directed by the patient's symptoms. This chapter will discuss the various radiologic findings in the following order: neuroradiology, thoracic radiology, musculoskeletal and abdominal radiology.

Neuroradiologic investigation heavily depends on computed tomography (CT) and magnetic resonance imaging (MRI). CT scan is usually the initial imaging modality; non-enhanced CT can provide information about the brain, ventricular size, hemorrhage, calcifications, mass, etc. The paranasal sinuses and skull base (e.g., temporal bones) are well evaluated by CT. Intravenous (IV) administration of contrast will give additional information about breakdown of the blood-brain barrier. Meningeal disease is often not apparent on non-enhanced CT. MRI examination is performed when the brain CT scan does not provide enough information or when the spinal cord is the suspected site of pathology. Unlike CT, which uses x-ray beam attenuation to generate imaging data, MRI utilizes the body's hydrogen atoms' response to a strong magnetic field and radiofrequency (RF) pulses. By altering RF pulses, different tissue signals can be generated. Pulse sequences that typically used are T1-weighted (T1WI), T2-weighted (T2WI), and proton density images (PDI). On T1WI, cerebrospinal fluid (CSF) is dark, and white matter is brighter than gray matter. On T2WI, CSF is bright, and white matter is darker than gray matter. On PDI, CSF is dark but not as dark as on T1WI, and white matter is darker than gray matter. In addition, images can also be obtained in axial, coronal and sagittal planes, etc. MRI is more sensitive than CT to small differences in tissue: normal or pathologic. *Lesions tend to be more conspicuous on T2WI than on T1WI.* Similar to CT, the use of IV paramagnetic contrast agents can increase the detection of lesions on T1WI by demonstrating breakdown of the blood-brain or blood-spinal cord barrier.

Although MRI is an excellent modality, its extremely strong magnetic field can injure patients if precautions are not taken. *Patients must be screened for potentially dangerous contraindicated conditions* such as pacemakers, MR-incompatible aneurysm clips, and ferromagnetic ocular foreign bodies. MRI is

also much more sensitive than CT to patient motion. Sedation may be required for patients unable to cooperate for MRI.

The roles of radiography and angiography in evaluating neurologic disease have become limited since the introduction of CT and MRI. Angiography, however, remains the definitive examination for evaluating suspected vascular abnormalities such as stenosis, aneurysm, and vasculitis. Recently MR angiography and CT angiography have also been used in such cases.

Radiography is the principal method for evaluating thoracic mycotic disease. Radiography is the most inexpensive and universally available imaging technique. It is both very sensitive and specific in demonstrating clinically important thoracic fungal disease. During a radiographic examination, x-rays enter the patient, and many exit the patient to enter a cassette containing a radiographic screen and film. The x-rays cause a fluorescent material on the surface of the screen to emit light, which exposes the radiographic film, creating an image. In the thorax, air-filled lungs have very limited ability to stop the transit of x-rays; hence lungs appear dark on radiographs. Soft tissues of the chest (mediastinum, hila, pleura, and chest wall) appear white on the radiograph, because they absorb a much greater proportion of the x-rays. Thoracic mycotic disease may create one or more foci of opacity within black lungs, alter the contours of the mediastinum and hila from adenopathy, or cause increasing opacity and widening of the pleural spaces from effusion.

In most cases, pertinent features are adequately displayed by radiographs to determine the extent and to monitor progress of mycotic disease and response to therapy. CT is usually the study of choice for further evaluation of patients. It has the advantages of greater contrast sensitivity than radiography and the ability to present the lungs, mediastinum, hila, and chest wall in cross-section. Rarely angiography, nuclear scintigraphy, or MR is necessary to give additional insight to radiographic and CT examinations. Through tagging of a radioactive substance to a variety of carrier substances that have affinity for normal anatomic structures or foci of disease, nuclear scintigraphy provides functional studies of the thorax (e.g., ventilation-perfusion scintigraphy and indium-labeled white blood cell scintigraphy). MR improves multiplanar presentation of normal and pathologic anatomy, may augment evaluating mediastinal and hilar structures, and may provide

a more sensitive evaluation. Radiographs are extremely useful in initial evaluation of skeletal pathology. Osseous lesion morphology is well demonstrated on radiographs. However, the extent of pathologic involvement of a bone is typically better determined with either CT or MRI. Bone marrow and soft tissue abnormalities are best depicted by MRI, whereas CT is best for evaluating cortical bone pathology or for the presence of calcium within a lesion. Ultrasonography is less useful in evaluating the skeletal lesions. Radionuclide studies with bone-avid agents are useful for establishing multiple sites of involvement and for evaluating for suspected osteomyelitis.

Investigation of abdominal pathology relies heavily on CT and ultrasonography. CT scans are typically performed with IV contrast to help identify lesions. Both modalities can be used to evaluate the abdominal viscera, but sonography is preferable to CT in evaluating patients with compromised renal function. Doppler sonography is useful to establish the presence or absence of flow through abdominal blood vessels, although angiography is still the definitive for vascular abnormalities. MRI of the abdomen is less often used.

## Neuroradiology of fungal infections

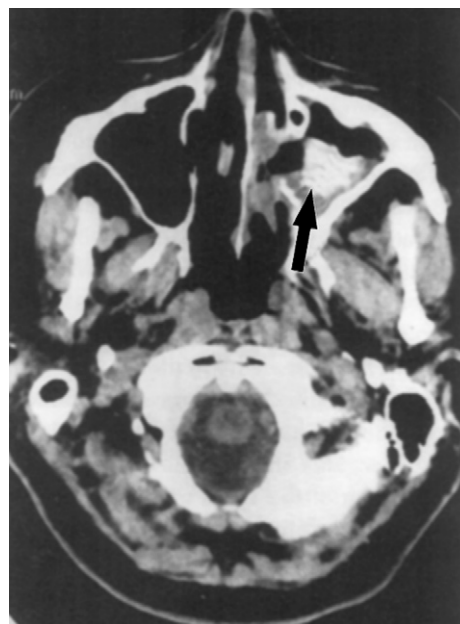
### Aspergillosis

*Aspergillus* is the most common fungus to involve the paranasal sinuses and four forms of sinus involvement have been described.<sup>1</sup> The two mucosal forms are fulminant invasive and chronic indolent sinusitis. The two extramucosal forms are non-invasive allergic sinusitis and aspergilloma (fungus ball). *The maxillary sinus is the most frequently involved site.* Chronic extramucosal fungal sinusitis develops as a saprophytic growth in retained secretions in a sinus cavity.<sup>2</sup> The radiographic findings are non-specific: mucosal thickening, sinus opacification, sinus wall erosion or sclerosis, or sinus expansion.<sup>2</sup> Fungus balls (Fig. 6-1) appear as polypoid soft tissue masses or as areas of sinus hyperdensity.<sup>3,4</sup> In 105 cases of paranasal sinus aspergillosis reported by Kopp et al.,<sup>3</sup> the most frequent appearance was homogeneous opacification of the maxillary sinus in 50% of the cases. Calcific densities (2–20 mm size) were present within opacified sinuses in 59% of Kopp's series.<sup>3</sup>

Mucosal thickening, masses within the sinus and osseous changes are better demonstrated with CT.<sup>4</sup> Zinreich et al.<sup>4</sup> found good correlation between the presence of hyperdensity within sinuses on CT and fungal sinusitis. However, thick pus, dessicated mucosal secretions, dystrophic calcifications, and hemorrhage can also appear dense on CT.<sup>2,4</sup> In addition, three of 25 patients were diagnosed after histopathologic examination. On MRI the inflammatory edema and cellular infiltrate of acute invasive fungal sinusitis will appear bright on T2WI and PDI. On T1WI these same regions will appear relatively hypointense. Similar signal changes can also be seen with allergic *Aspergillus* sinusitis. The presence of a fungus ball or desiccated secretions or both in chronic fungal sinusitis results in T1 relatively hypointense and T2 markedly hypointense regions within the affected sinuses, and can mimic a normally aerated sinus. But T1WI or CT images will reveal the extent of disease (Fig. 6-2). The decreased MR signal of fungal concretions has been attributed to the presence of calcium, iron and manganese.<sup>4,6</sup>

Invasive *Aspergillus* sinusitis may be seen at presentation with extension into adjacent soft tissues of the face, orbit, or intracranial cavity (Fig. 6-3). Orbital invasion may result in erosion of the orbit walls, subperiosteal phlegmon, inflammatory edema, and orbital abscess.<sup>5</sup> An inflammatory phlegmon generally demonstrates diffuse contrast enhancement on CT or MRI, whereas an abscess typically has peripheral enhancement around a necrotic center. Bone destruction is best evaluated with CT and soft tissue involvement is best demonstrated with multiplanar MR. Involvement of the optic nerve can be seen as enlargement or abnormal enhancement using either CT or MRI. Edema of the optic nerve is better demonstrated with T2-weighted MR images than with CT. Optic nerve enhancement is best appreciated by use of fat-suppressed T1-weighted coronal MR images. The bright T1 signal of enhanced extraocular muscles on fat-suppressed images is to be expected and should not be confused with inflammation or infection. When involved, they are enlarged with an abnormal increased T2 signal.

Invasion of the cavernous sinus can occur from adjacent paranasal sinus disease or through the orbital apex.<sup>6</sup> Non-enhancement of the cavernous sinus on contrast CT or MRI is indicative of sinus thrombosis. When the cavernous sinus is involved without thrombosis, it appears enlarged. Intracranial extension of *Aspergillus* can occur from the sinuses or through the orbital apex. The radiographic signs of early invasive CNS aspergillosis can be subtle, such as an intracranial focus of minimal enhancement adjacent to an involved sinus. If untreated, the initial focus of enhancement may go on to develop into abscesses. Intracranial granuloma formation has also been reported secondary to invasive sinus aspergillosis.<sup>7</sup> Cerebral aspergillosis, however, usually occurs by



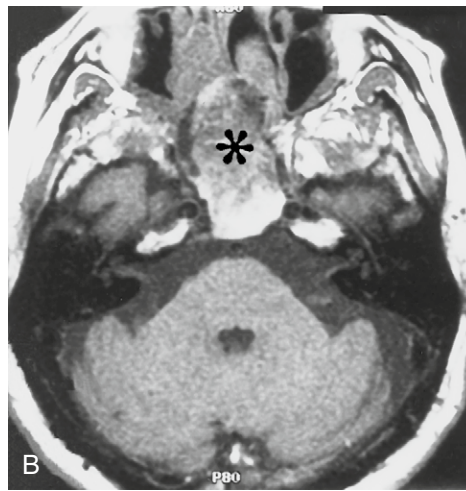
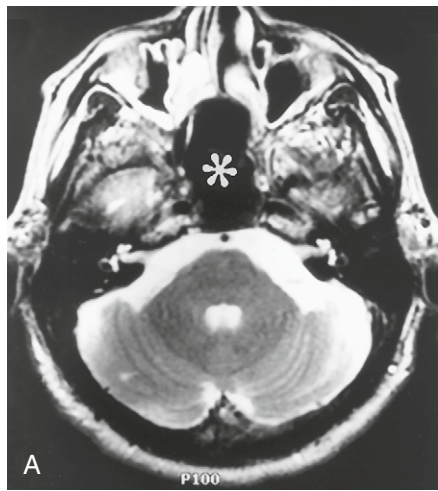
**Figure 6-1** *Aspergillus* fungus ball. Axial CT scan through the maxillary sinuses shows a calcified fungus ball (arrow) in the left maxillary sinus. The thicker walls of the left maxillary sinus are indicative of chronic inflammatory disease.



means of hematogenous spread from a pulmonary focus.<sup>8</sup> Imaging findings of intracranial aspergillosis typically include multifocal cerebral hemispheric lesions, with hemorrhage in approximately 25% of lesions.<sup>9</sup> CNS aspergillosis can result in meningitis, meningoencephalitis, granuloma, brain abscess, or infarction.<sup>10</sup> Isolated *Aspergillus* meningitis is rare. Meningitis is frequently difficult to detect with CT or MRI. On CT, abnormal increased density in the basal cisterns, especially if there is contrast enhancement of the cisterns, is indicative of meningitis.<sup>11</sup> MRI is more sensitive than CT in detecting abnormal cisternal and sulcal contrast enhancement, especially if the brain is imaged in multiple planes. Cerebritis or

infarction may initially have only subtle decreased density on CT or increased signal on T2WI and fluid-attenuated inversion recovery (FLAIR)-MR images.

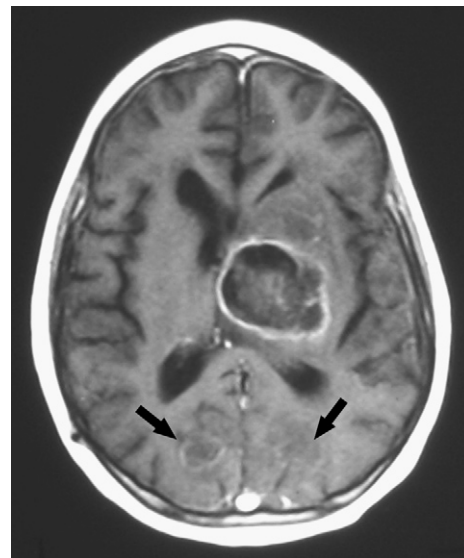
Ashdown et al<sup>12</sup> described three patterns of cerebral aspergillosis in immunocompromised patients: infarctions, abscesses, and dural enhancement. Multiple areas of cortical and subcortical hypodensity on CT and hyperintensity on T2WI were consistent with infarctions. Enhancement was often minimal. When infarctions were hemorrhagic, they exhibited increased density on CT and increased T1 signal on MRI. Abscesses appeared as multiple ring-enhancing lesions often at the gray-white matter junction (Fig. 6-4). Most of the



**Figure 6-2** *Aspergillus* sphenoid sinusitis. (A) Axial T2-weighted image through the maxillary and sphenoid sinuses shows marked hypointensity (white asterisk) in the sphenoid sinus which mimics normal aeration. (B) Axial T1-weighted image at the same level demonstrates that the sphenoid sinus is full of soft tissue (black asterisk) and is not normally aerated.



**Figure 6-3** Invasive aspergillosis. Axial CT scan shows an *Aspergillus* mass filling the left orbit posteriorly. There is involvement of the adjacent ethmoid sinuses and erosion of the orbit medial wall (arrowhead).



**Figure 6-4** *Aspergillus* brain abscesses. Axial T1-weighted image with gadolinium shows a large thalamic astrocytoma and faint ring-enhancing *Aspergillus* abscesses in the occipital lobes (arrows) (With permission from Rastogi H, et al. The posttherapeutic cranium. In: Jinkins JR (ed) Posttherapeutic Neurodiagnostic Imaging. Lippincott-Raven Publishers, New York, 1997, p.3.)

abscesses had irregular thick rim enhancement. The third pattern was enhancement of dura and of lesions in the adjacent paranasal sinuses, orbit, or skull.

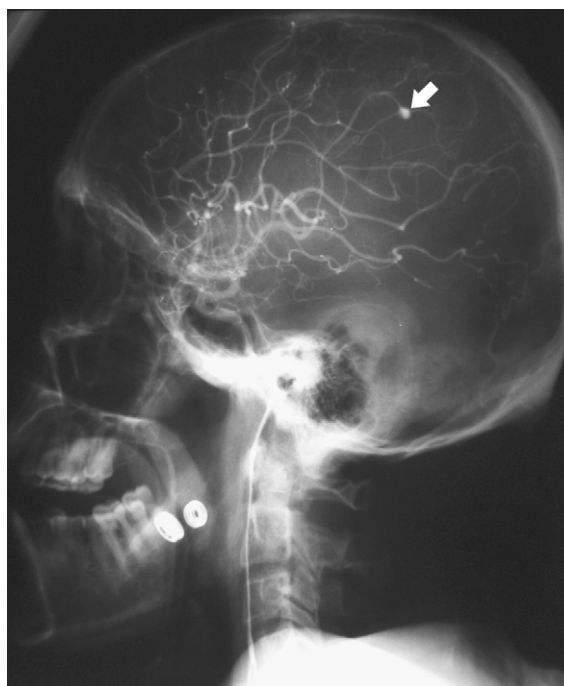
*Aspergillus* has a propensity to invade blood vessels,<sup>5</sup> which can lead to thrombosis, hemorrhage, and formation of mycotic aneurysms. Thrombosis, especially of larger vessels, can be detected on MRI as increased signal within a vessel and loss of the expected flow void. On CT, thrombosed vessels fail to enhance after IV contrast. The angiographic findings of vascular invasion include areas of irregular narrowing, dilatation,<sup>11</sup> aneurysms<sup>13</sup> (Fig. 6-5) and thrombosis. MR and CT angiography in some cases can also be used to demonstrate the vascular changes of aspergillosis. Okafuji et al described a case with CT and MRI showing multiple corticomedullary lesions with central enhancement contiguous with markedly dilated adjacent cortical vessels. These corresponded with hemorrhagic infarctions and dilated cortical veins thrombosed with *Aspergillus* hyphae.<sup>14</sup>

Three different patterns of craniocerebral involvement have been described in immunocompetent hosts which correlate with clinical outcome. Type I, intracerebral aspergillosis with worst clinical outcome; type II, intracranial extradural with intermediate outcome, and type III, orbital and skull base aspergillosis with good outcome.<sup>20,21</sup> The imaging appearance depends on the immunologic status of the patient and the age of the lesion.<sup>2,22</sup> In general, the lesional enhancement is stronger in immunocompetent hosts compared to immunocompromised hosts.<sup>15</sup>

*Aspergillus* infection of the spine is rare but intervertebral disk infection, vertebral osteomyelitis, epidural abscess and granuloma, and spinal cord infarction have been reported.<sup>24,25</sup> Spinal involvement can be from local or hematogenous spread. Radiographs of the spine can reveal disk space narrowing and destruction of the vertebral bodies (Fig. 6-6). Radionuclide bone scans will demonstrate abnormal increased activity within infected vertebrae. The extent of bone destruction is better depicted by CT than by radiographs. MRI will show early bone marrow disease and soft tissue abnormalities as foci of increased T2 signal and decreased T1 signal. Granulomas, abscesses, and osteomyelitis will typically enhance with IV contrast. Lesions of the spinal cord are rare.<sup>16</sup> If MRI is not available or is contraindicated for a patient, then myelography with subsequent CT can be used to identify extradural and intramedullary changes.

## Blastomycosis

Osseous involvement in systemic blastomycosis is seen in 10–60% of cases,<sup>17</sup> most frequently the thoracic and lumbar spine.<sup>17</sup> In children the intervertebral disks are typically affected.<sup>17</sup> The lesions of blastomycotic osteomyelitis are typically osteolytic with minimal surrounding reactive sclerosis.<sup>18</sup> In the spine, narrowing of the intervertebral disks and paraspinal masses are common<sup>18</sup> (Fig. 6-7). Vertebral collapse occurs late in the disease course.<sup>18</sup> Radionuclide bone scanning demonstrates increased activity.<sup>27,28</sup> CT can demonstrate bone destruction even when radiographs are normal.<sup>18</sup> On MRI, osseous lesions will have decreased T1 and increased T2 signal compared with normal fatty marrow. Contrast-enhanced MR can help distinguish granulomatous reaction from true abscesses.<sup>17</sup> An abscess will have a necrotic center with peripheral enhancement. Granulomatous reaction will have

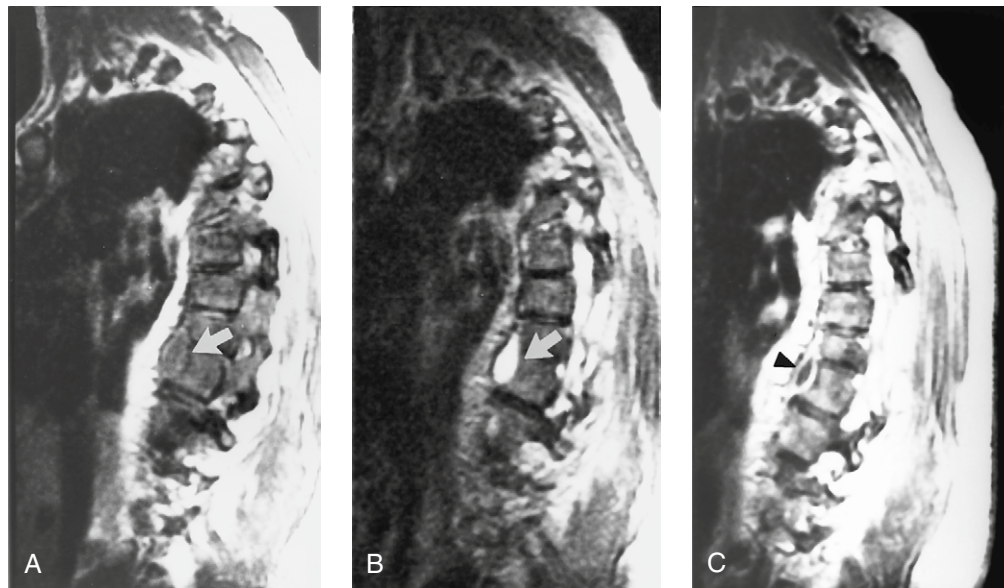


**Figure 6-5** Mycotic aneurysm unidentified organism. Lateral view of a right carotid arteriogram shows a mycotic aneurysm (white arrow) of a posterior branch of the middle cerebral artery.



**Figure 6-6** *Aspergillus* osteomyelitis/diskitis. Lateral radiograph of the lumbar spine shows narrowing of the L2–L3 disk. There is erosion of the anterior aspect of the L2 inferior endplate and L3 superior endplate (arrow).

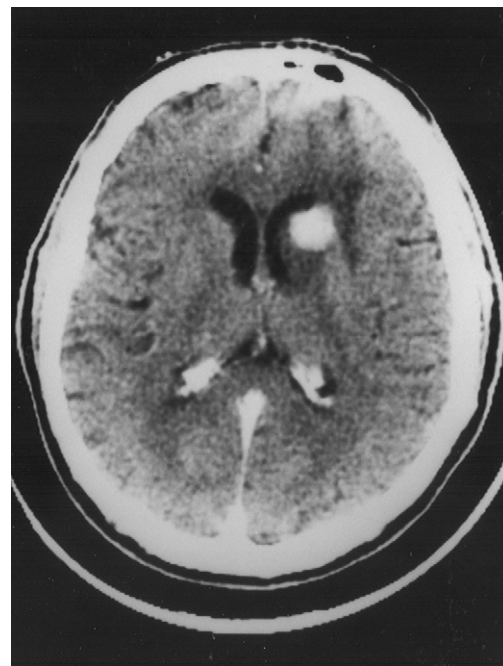
a more diffuse enhancement pattern. CT, MRI or both can be used to define the extent of paraspinal soft tissue involvement. MR is better than CT to detect and delineate the extent of intraspinal disease, epidural granulomas, abscesses, intramedullary granulomas or edema from compressive lesions.



**Figure 6-7** Blastomycosis paravertebral abscess. (A) Thoracic spine T1-weighted image shows a paraspinal blastomycosis abscess which has an isointense center and a slightly hyperintense periphery (white arrow). (B) Sagittal T2-weighted image shows hyperintense abscess (white arrow). (C) Sagittal T1-weighted image with gadolinium shows rim enhancement of the abscess (arrowhead).

Blastomycosis can also involve the cranium with lytic lesions.<sup>19</sup> High-resolution CT will demonstrate bone destruction of the skull base and soft tissues. On MRI, involved bone will have decreased T1 and increased T2 signal. MR can show the extent of bone marrow involvement and intracranial extension better than CT, especially in the region of the skull base.<sup>20</sup> Blackledge et al described an unsuspected case of petrous apex blastomycosis in a man with a 3-week history of progressive hearing loss, and facial paralysis mimicking a neoplasm.<sup>23</sup>

Buechner and Clawson<sup>21</sup> found only nine patients (4.5%) with CNS involvement in 198 cases of blastomycosis. Patients with CNS blastomycosis can present with acute or chronic meningitis or mass lesions of the brain or spinal cord.<sup>22</sup> Blastomycotic meningitis is difficult to diagnose unless the patient has obvious systemic blastomycosis elsewhere.<sup>23</sup> Kravitz et al<sup>23</sup> reported three patients with chronic blastomycotic meningitis with only hydrocephalus on CT. Friedman et al reported a case of meningoencephalitis with progressive enhancement of basal meninges with involvement of bilateral basal ganglia and thalami on MR.<sup>24</sup> Cerebral lesions may be solitary or multiple. Roos et al<sup>25</sup> reported four cases that on CT scans had single lesions that were isodense to slightly hyperdense that enhanced homogeneously with surrounding edema (Fig. 6-8). Angtuaco et al<sup>20</sup> reported multiple solid enhancing lesions seen on MRI. Imaging findings are similar to other granulomatous diseases and biopsy is invariably needed to make histologic diagnosis.<sup>28,31</sup>



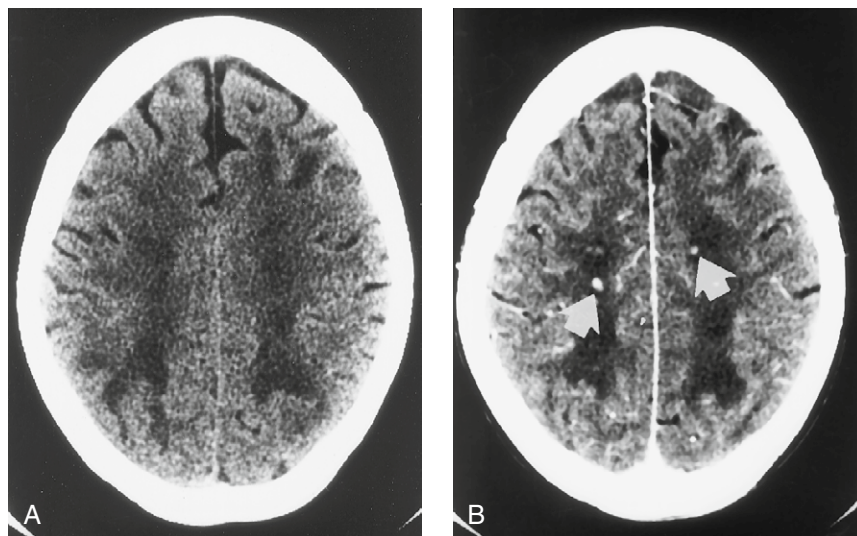
**Figure 6-8** Cerebral blastomycosis in a 22-year-old man with a 1-week history of headache. CT scan shows an enhancing lesion adjacent to the left frontal horn; there is surrounding low-density edema.

## Candidiasis

Central nervous system infection with *Candida* is seen in approximately half of autopsied patients with systemic candidiasis.<sup>26</sup> The CNS is usually infected through hematogenous dissemination. Cerebral candidiasis usually results in multiple microabscesses (Fig. 6-9) or granulomas and rarely in meningitis.<sup>27</sup> In addition, fungus ball, candidal ependymitis, macroabscesses, infarction, mycotic aneurysm, and demyelination have also been reported.<sup>26</sup> The spinal involvement can range from spondylodiskitis to myeloradiculitis.

Coker<sup>28</sup> reported two infants with *Candida* meningitis and ventriculitis that showed progressive development of hydrocephalus. On contrast-enhanced CT the ventricles had enhancing trabeculations and periventricular enhancement. Cranial ultrasonography demonstrated dilated ventricles with echogenic debris and periventricular cavitation, poorly defined foci of parenchymal echogenicity (cerebritis), and multiple cortical hypoechoic areas (granulomatous abscesses).<sup>35,36</sup> Multiple large ring-enhancing lesions with edema and hydrocephalus from *Candida* abscesses were reported by Chaabane et al.<sup>29</sup>





**Figure 6-9** *Candida* micro abscesses. (A) Non-enhanced CT scan shows abnormal low density within the white matter. (B) Contrast CT scan shows punctate enhancing candidal microabscesses (white arrows). (Case courtesy of Dr Richard Dahlen, San Antonio, TX.)

Calcifications have been reported as the end-stage of *Candida* lesions after therapy with amphotericin B.<sup>27</sup> Radionuclide single photon emission CT (SPECT) scanning with thallium-201 has shown abnormal radionuclide uptake in cerebral lesions and correlated with lesions seen on contrast-enhanced CT. After treatment with amphotericin B, most of the abnormal Tl-201 uptake and the enhancement on CT disappeared.

Vascular invasion by *Candida* can result in thrombosis, vasculitis, and mycotic aneurysms.<sup>30,36</sup> Thrombosis leads to cerebral infarction, which may become hemorrhagic. Serial arteriography has shown an increase in the size and number of the mycotic aneurysms.<sup>26</sup> Rupture of mycotic aneurysm results in subarachnoid hemorrhage.

Bone infection by *Candida* is rare.<sup>30</sup> Edwards et al<sup>31</sup> reported three patients with *Candida* vertebral osteomyelitis with lytic lesions. Cervical osteomyelitis with C6–C7 vertebral destruction, loss of disk space, subluxation<sup>32</sup> and epidural abscess has been described.<sup>33</sup>

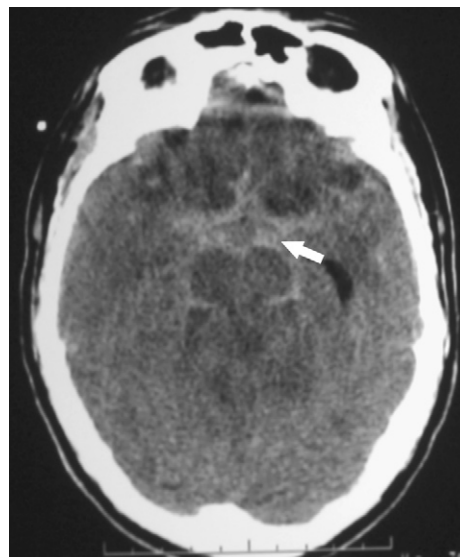
MRI identifies the level and extent of spinal disease and helps evaluation of the thecal sac and its contents. *Bright T2 signal within the cord, though non-specific, may represent edema, ischemia or gliosis, and is a bad imaging sign.* Microbiologic diagnosis of spondylodiskitis requires CT-guided biopsy. Without antifungal treatment the *Candida* vertebral osteomyelitis can progress to vertebral collapse and neurologic compromise within 3–6 months of symptom onset.<sup>41</sup>

*Candida* vertebra has been reported in an immunocompetent girl with factor X deficiency due to an infected cannula.<sup>34</sup>

## Coccidioidomycosis

Dissemination of *Coccidioides* occurs in less than 1% of infections but when it does happen, the CNS is involved in one-third to three-quarters of cases.<sup>35</sup> *Meningitis is the most frequent CNS manifestation of disseminated Coccidioides infection.*<sup>44,45</sup> Sobel et al<sup>36</sup> described four patterns in 32 patients: leptomeningitis alone, leptomeningitis with cerebritis, leptomeningitis with cerebritis and infarcts, and multiple granulomas.

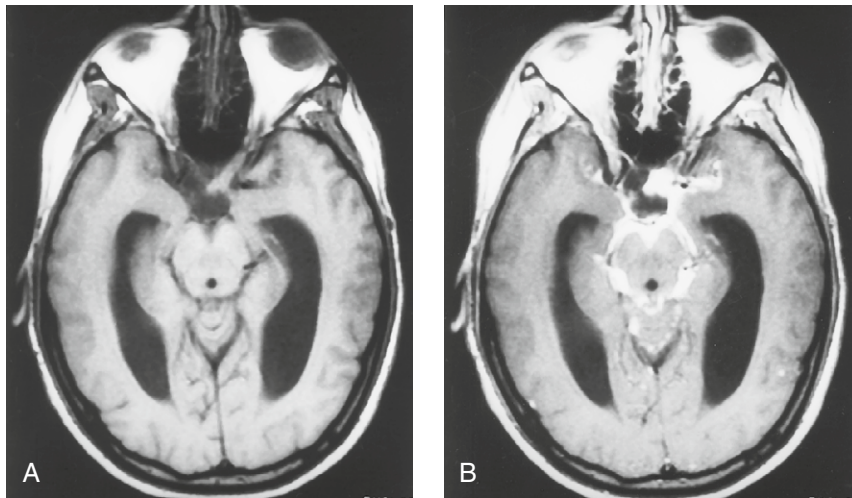
Arsura et al,<sup>37</sup> in their study of 62 patients with coccidioidal meningitis, found abnormalities in 76% and 41.6% of patients on MRI and CT scan respectively. *Hydrocephalus*



**Figure 6-10** *Coccidioides* meningitis. Axial non-enhanced CT scan shows abnormal increased density in the suprasellar cisterns caused by dense inflammatory exudate (white arrow).

(51.6%), *basilar meningitis* (46.8%) and *cerebral infarctions* (38.7%) were the most common findings (Fig. 6-10). Dublin and Phillips<sup>35</sup> also described a high incidence of hydrocephalus (80%) and leptomeningeal signs (obliteration, distortion, and/or increased density, enhancement within the basal cisterns) (67%) on CT. White matter abnormalities were seen in 40%; less frequent were ventriculitis (enhancement of the ventricular ependymal lining), focal granuloma (nodular enhancing mass lesion), and deep gray matter lesions (abscesses or infarcts). MRI with diffusion-weighted sequence showed a higher incidence of infarction than CT.<sup>37</sup> The presence of hydrocephalus and hydrocephalus co-existing with infarction was associated with increased mortality rates.<sup>37</sup>

The MRI findings of coccidioidomycosis meningitis in 12 patients were described by Wrobel et al.<sup>38</sup> Precontrast MRI demonstrated abnormal increased signal within the

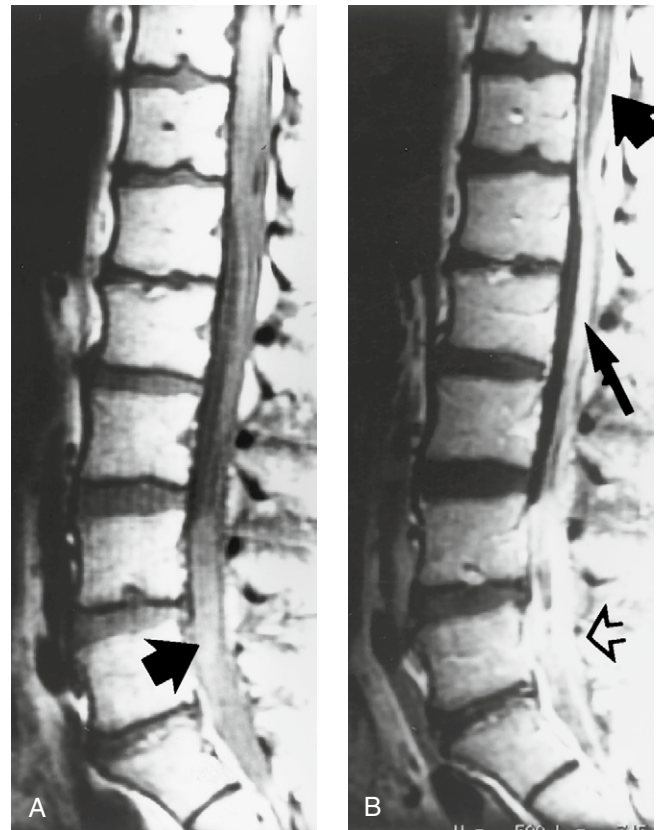


**Figure 6-11** *Coccidioides* meningitis. (A) Axial T1-weighted image shows hydrocephalus and enlarged temporal horns. (B) Axial T1-weighted image with gadolinium shows intense abnormal meningeal enhancement of the suprasellar cisterns, around the midbrain, and superior vermis. (Case courtesy of Dr Richard Dahlen, San Antonio, TX.)

subarachnoid spaces on PD images. Abnormal meningeal enhancement of the basal cisterns, sylvian fissures, and interhemispheric fissure was present in 58% of their cases (Fig. 6-11). Varying degrees of hydrocephalus were present in seven of 12 patients. Periventricular increased T2 signal was not prominent in any of their patients. Focal areas of parenchymal increased T2 signal suggestive of edema or ischemia or infarction were present predominantly in the white matter in four of the patients. Cortical as well as deep infarcts are seen in some patients.<sup>45,47</sup> Pathologically, the areas of abnormal MRI enhancement represent focal collections of organism.<sup>39</sup>

Vascular involvement is frequently found at autopsy typically affecting small arteries and arterioles.<sup>36</sup> In Sobel's series infarcts were frequently multiple, occurring commonly in the basal ganglia, thalamus, and white matter. Kleinschmidt-DeMasters et al reported *C. immitis* ventriculitis with venulitis and widespread dural and cerebral venous thrombosis in an AIDS patient.<sup>61</sup>

Osseous involvement was reported in 10–50% of cases of disseminated coccidioidomycosis. Multiple lytic lesions of the skull demonstrated increased activity on radionuclide bone scan.<sup>40</sup> *The spine is the most common site of bone infection.* Spine lesions are lytic and can either have a poorly defined margin or appear “punched out.”<sup>40</sup> Dalinka and Greendyke<sup>41</sup> reported 17 spinal lesions in seven patients. Multiple, non-contiguous spinal lesions were present with associated involvement of other bones. Collapse of the vertebral bodies occurred late in the disease course. The disks were relatively spared and were involved late in the disease. Involvement of the thoracic spine was usually associated with a paraspinal abscess. Intraspinal disease can occur as epidural extension of osseous lesions and result in spinal cord compression<sup>42</sup> or meningeal infection.<sup>38</sup> Wrobel and Rothrock<sup>43</sup> reported two patients with anterior spinal artery syndrome secondary to extensive cervical subarachnoid involvement with *Coccidioides immitis*. MRI demonstrated thick, abnormally enhancing meningeal granulation tissue with flattened and compressed cervical cord. Spinal arachnoiditis can show clumping of nerve roots, enhancement of nerve roots, thickening of the meninges, abnormal enhancement of the meninges, and abnormal increased T1 and PD signal of the CSF on MRI (Fig. 6-12).

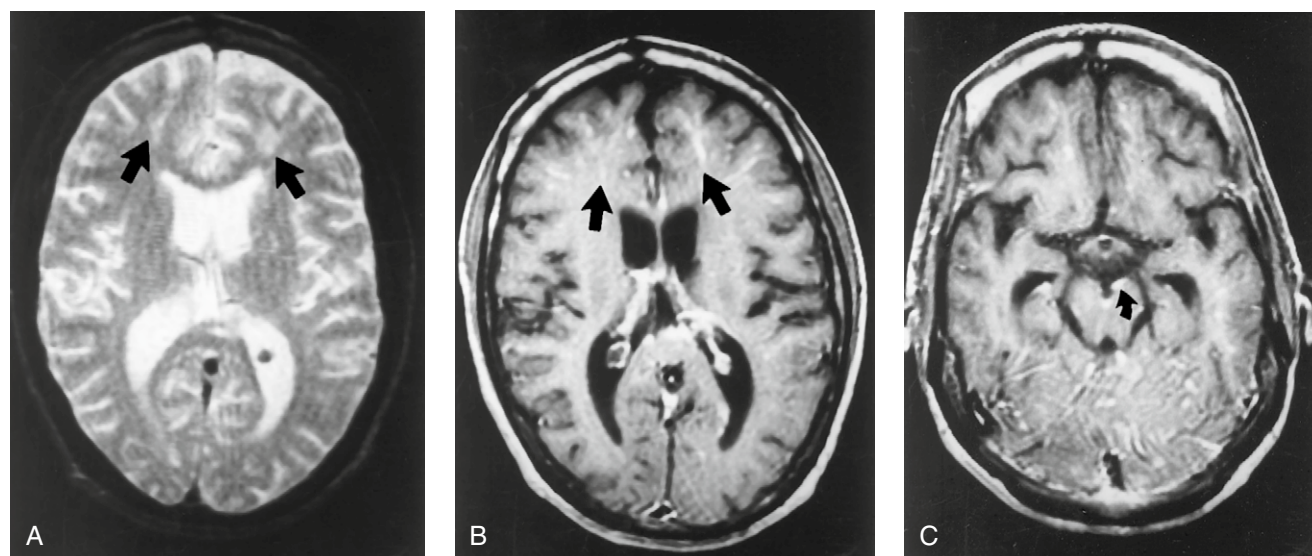


**Figure 6-12** Spinal *Coccidioides* meningitis. (A) Non-enhanced sagittal T1-weighted image shows abnormal increased signal in the lower lumbosacral thecal sac (arrow). (B) Sagittal T1-weighted image with gadolinium shows intense abnormal enhancement coating the conus medullaris (arrow) and the cauda equina (long arrow). The lower lumbosacral thecal sac shows abnormal diffuse enhancement (open arrow).

## Cryptococcosis

*Cryptococcus* is very neurotropic and can involve the CNS as meningitis, meningoencephalitis, or cryptococcal masses. *Meningitis is the most common clinical presentation.* In “normal” hosts, *Cryptococcus* elicits a granulomatous reaction.<sup>44</sup>





**Figure 6-13** Cryptococcal meningoencephalitis. (A) Axial T2-weighted image shows abnormal increased signal in the frontal lobes (arrows). (B) Axial T1-weighted image with gadolinium shows abnormal enhancement within areas of cerebritis in the frontal lobes (arrows). (C) Axial T1-weighted image with gadolinium shows abnormal meningeal enhancement around the midbrain (curved arrow).

Immunocompromised patients may mount little, if any, inflammatory reaction.

In the series of intracranial cryptococcosis reported by Popovich<sup>44</sup> and by Cornell,<sup>45</sup> slightly more than 40% of patients had no abnormalities on CT. In the 12 cases of intracranial cryptococcosis reported,<sup>45</sup> the most common abnormality on CT was hydrocephalus, which was present in 58% of their cases. However, only 9% of the 35 patients with intracranial cryptococcosis described by Popovich<sup>44</sup> had hydrocephalus. Hydrocephalus is thought to result from adhesions secondary to chronic meningeal inflammation that produce CSF obstruction. In the Popovich series, 80% of the patients had AIDS. CT scans in the 12 non-AIDS patients reported by Cornell revealed intense meningeal enhancement, obliteration of the fourth ventricle secondary to cerebritis, and enhancing intraventricular granuloma. Takasu et al<sup>46</sup> reported the case of an HIV-negative man with cryptococcal meningoencephalitis with multiple enhancing lesions on MRI. T2-weighted images showed abnormal increased signal in the frontal lobe and adjacent to the fourth ventricle with resolution on repeat MRI. Riccio<sup>47</sup> reported the CT and MR findings of a non-AIDS patient with biopsy-proven multiple cryptococcal microabscesses. Non-contrast CT demonstrated multiple small calcifications and slight ventricular dilatation. The MR revealed numerous scattered nodules of abnormal enhancement, most of which were in sulci, and slight meningeal enhancement adjacent to the pons (Fig. 6-13).

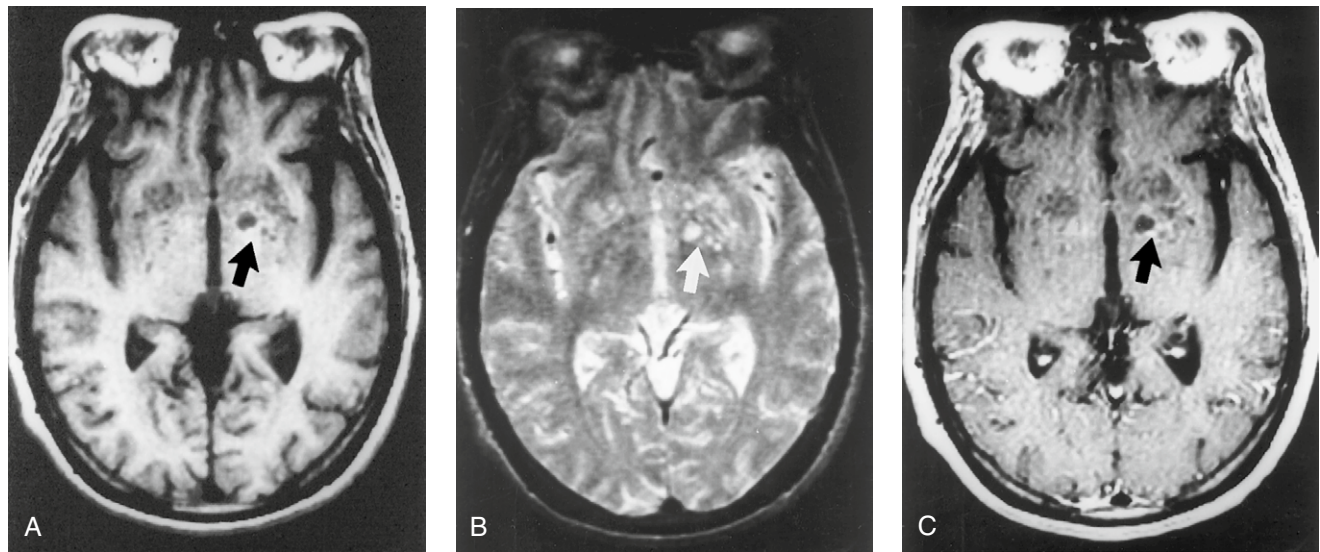
In the series of 11 non-AIDS patients reported by Chan et al,<sup>48</sup> two patients had unusual findings on CT: a cryptococcal cyst of the pituitary gland, and a cyst of the posterior fossa. The cause was unsuspected until after examination of the cyst fluid. Kanter et al<sup>49</sup> reported a non-AIDS patient whose CT showed multiple solid and ring-enhancing lesions, some with surrounding edema. With a presumptive diagnosis of metastatic disease, steroid and radiation therapy was started. A repeat CT scan showed that the enhancing lesions had almost completely disappeared. After 2 weeks of improvement, the patient began to deteriorate and subsequently died. Autopsy revealed diffuse involvement of the

leptomeninges by *Cryptococcus*. Garcia et al<sup>50</sup> reported two non-AIDS patients with intracranial cryptococcosis that on CT demonstrated multiple round hypodense non-enhancing lesions in the basal ganglia and thalami. These lesions histologically consisted of cavities (dilated Virchow-Robin spaces) filled with a gelatinous material that contained numerous organisms with thick capsules. Garcia et al coined the term “gelatinous pseudocysts” (Fig. 6-14) for these lesions, because there was no membrane between the cavities and the adjacent brain.

Mathews et al<sup>51</sup> correlated the CT and MR findings of intracranial cryptococcosis with autopsy studies and concluded that MR detects more lesions than CT but that both imaging modalities missed more than 50% of the lesions that are present at autopsy. They also reported that the small foci of increased T2 signal seen in the basal ganglia were more often the result of small intraparenchymal cryptococcoma. The gelatinous pseudocyst and parenchymal cryptococcoma in immunocompromised patients typically show lack of diffusion restriction, poor to absent enhancement on MRI and negative thallium SPECT.<sup>62,63</sup> However, immunocompetent patients, presumably due to retained ability to mount intense inflammatory response, may instead show restricted diffusion, enhancement and false-positive thallium SPECT. A true-positive SPECT is suggestive of tumors as hypercellularity and breakdown of the blood–brain barrier leads to increased thallium uptake. The immunocompetent host who has an ability to mount intense inflammatory response causes breakdown of the blood–brain barrier (hence enhancement), increased cellularity and presumably increased viscosity of the necrotic material (hence restricted diffusion, seen as bright signal).<sup>62,63</sup> These imaging findings and the patient’s non-suspecting immunocompetent status can confound diagnosis.

Cryptococcal infection of the spine is rare, but osteomyelitis with epidural extension,<sup>52</sup> arachnoiditis,<sup>53</sup> and intramedullary granuloma have all been reported. Cure<sup>52</sup> reported the MR findings of cryptococcal spondylitis in an HIV-negative patient. Increased T2 signal was present in vertebral bodies T9–T10. The paravertebral infection extended into the epidural space,





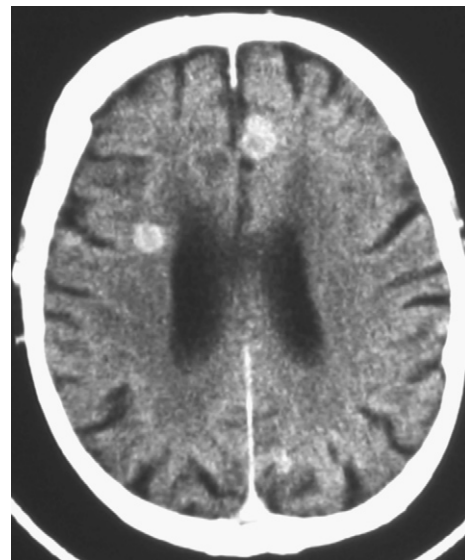
**Figure 6-14** Cryptococcal gelatinous pseudocysts. (A) Axial T1-weighted image shows hypointense dilated Virchow-Robin spaces (*arrow*), gelatinous pseudocysts. (B) Axial T2-weighted image shows hyperintense dilated Virchow-Robin spaces (*white arrow*). (C) Axial T1-weighted image with gadolinium shows minimal enhancement of the gelatinous pseudocysts (*arrow*).

with resultant spinal cord compression. Gadolinium-enhanced fat-suppressed T1WI demonstrated abnormal enhancement not only of T9 and T10 but also of the T8 vertebral body and the T9–10 disk, which had not demonstrated abnormal signal on T2-weighted images. Stein et al<sup>53</sup> reported five patients with fungal spinal leptomeningitis, four cases secondary to *Cryptococcus* and one caused by *Aspergillus*. Three of the patients with cryptococcal arachnoiditis had myelograms that revealed incomplete or complete block. At surgery, the meninges were thickened, causing cord compression in all cases. Histologic examination of the abnormal meninges demonstrated *Cryptococcus*. MR examination of spinal arachnoiditis can show clumping of nerve roots, enhancement of nerve roots, thickening of the meninges, abnormal enhancement of the meninges, and abnormal increased T1, PD and FLAIR signal of the CSF. If the spinal cord is compressed, edema will appear as increased T2 signal. With disruption of the blood–spinal cord barrier by infarction or an intramedullary infection, enhancement of the affected cord may occur.

## Histoplasmosis

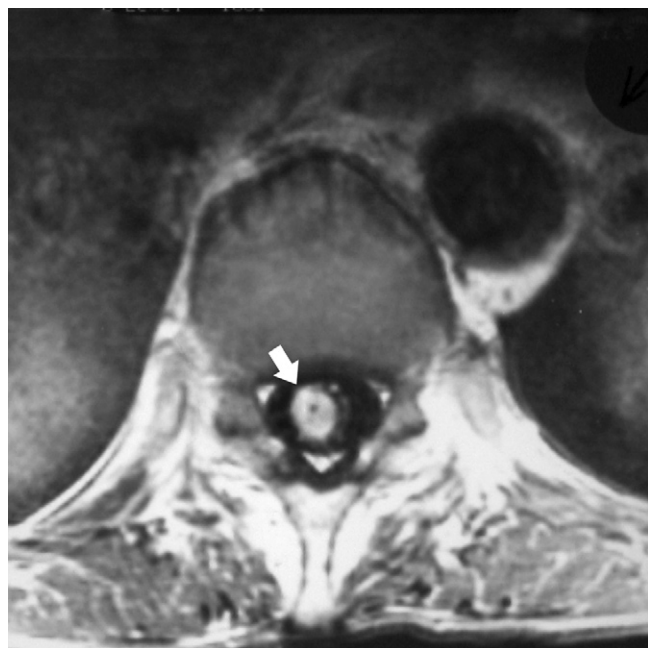
CNS involvement by *Histoplasma* is very rare except in cases of disseminated histoplasmosis. Autopsy series have shown CNS involvement in approximately 25% of cases of disseminated histoplasmosis but neurologic symptoms were present only in one-quarter of patients.<sup>54</sup> In a literature review of 77 cases of CNS histoplasmosis by Wheat et al,<sup>54</sup> approximately 65% had chronic meningitis, 25% had cerebral mass and less than 5% had cerebritis or spinal cord lesions.

Most CT and MR descriptions of CNS histoplasmosis have been reports of either solitary or multiple mass lesions (Fig. 6-15).<sup>55,56</sup> Walpole et al<sup>55</sup> reported a case of cerebral histoplasmosis with multiple ring-enhancing lesions with peripheral edema on CT that subsequently calcified. Dion et al<sup>56</sup> described a thalamic ring-enhancing lesion on CT that had a hypointense rim with slightly hyperintense center on PD MR images. Rivera



**Figure 6-15** Cerebral histoplasmosis. Enhanced brain CT scan shows multiple ring-enhancing *Histoplasma* lesions.

et al<sup>57</sup> described a 9-year-old patient with an initial CT showing only communicating hydrocephalus, treated with a ventriculoperitoneal shunt. Four years later the CT demonstrated a ring-enhancing lesion adjacent to the third ventricle, subependymal enhancement of the right lateral ventricle, and worsening hydrocephalus. Zalduondo et al<sup>58</sup> reported a case showing thick leptomeningeal enhancement at the base of the brain which involved the fifth cranial nerve on MR. T2WI showed communicating hydrocephalus and a round hyperintense thalamic lesion that did not enhance with contrast. CT performed one week later showed multiple confluent hypodense regions in the basal ganglia and subcortical white matter. At autopsy, the areas of hypodensity on CT in the thalamus and basal ganglia were due to cerebritis. Early cerebritis may not show contrast enhancement



**Figure 6-16** Intramedullary spinal cord *Histoplasma* granuloma. Axial T1-weighted image with gadolinium shows an enhancing granuloma within the substance of the thoracic cord (white arrow).

and can simulate infarction. Vasculitis was present and was thought to be the cause of the cerebritis. Livas et al<sup>59</sup> described multiple cerebral and spinal cord ring-enhancing *Histoplasma* lesions on MR which showed resolution after completion of therapy. Bazan et al<sup>60</sup> reported a case of intramedullary spinal cord histoplasmosis with multiple solid enhancing granulomas and edema in the conus medullaris (Fig. 6-16).

## Hyalohyphomycosis

Among the agents of hyalohyphomycosis, *Fusarium* and *Penicillium* have been reported to involve the CNS. Huang and Harris<sup>61</sup> reported a case in a leukemic patient who at autopsy had disseminated cerebral and pulmonary penicilliosis with vascular invasion, thrombosis, and infarction. Steinberg et al<sup>62</sup> reported a case of *Fusarium* brain abscess in the head of the right caudate nucleus with a slightly hyperdense rim with minimal edema that enhanced intensely on contrast-enhanced CT. A repeat CT after aspiration of the abscess demonstrated new ventricular dilatation and enhancement of the ventricular wall, suggesting ependymitis.

## Paracoccidioidomycosis

Initially, CNS involvement by *Paracoccidioides* was thought to be rare, but subsequent studies have revealed a frequency of CNS involvement ranging from 0.6% to 27.3%.<sup>63</sup> The disease, mostly affecting adult males, is endemic in subtropical areas of Central and South America;<sup>64</sup> the highest incidence occurs in Brazil. De Almeida et al reported a 4 cm extraaxial paracoccidioidal mass attached to the undersurface of the tentorium that compressed the cerebellum.<sup>64</sup> They also stated that *Paracoccidioides* produced three types of CNS lesions: meningoencephalitis, parenchymal granulomas, and isolated masses. The granulomatous form is the most common.<sup>65</sup> Based on CT



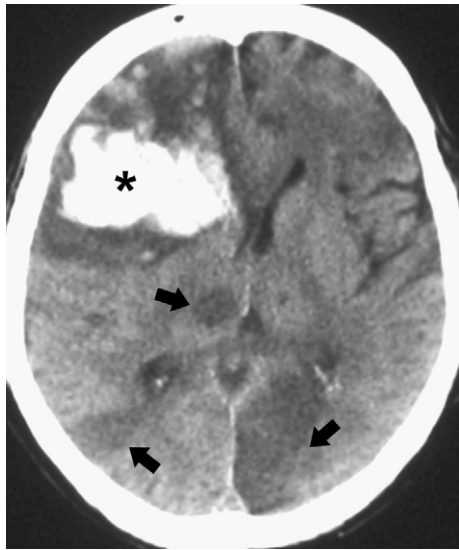
**Figure 6-17** Phaeohyphomycosis: *Bipolaris* invasive sinusitis. Coronal non-enhanced CT scan shows hyperdense fungal masses (asterisks) in the right maxillary and ethmoid sinuses. There is erosion of the floor of the right anterior cranial fossa (arrow) and remodeling of the right orbit medial wall.

scan findings, Elias et al<sup>66</sup> identified four imaging patterns: low-density lesion with ring enhancement, lesion with calcification and ring enhancement, multiloculated low-density lesion with ring enhancement, and diffuse subarachnoid enhancement. The MRI showed leptomeningeal enhancement in one and heterogeneous lesion with ring enhancement in two patients. Gasparetto et al<sup>67</sup> found ring-like contrast enhancement in 94%, perilesional edema in 82% of parenchymal lesions and hydrocephalus in 41% on CT.<sup>67</sup>

## Phaeohyphomycosis

Adam et al<sup>68</sup> reported nine cases of phaeohyphomycosis, three of which were examples of sinusitis caused by the genera *Alternaria*, *Bipolaris* and *Exserohilum*. Rinaldi et al<sup>68</sup> reported five cases of sinusitis caused by *Curvularia* in immunocompetent patients. Radiographs revealed either sinus opacification or soft tissue masses in the sinuses. CT scan confirmed the radiographic findings and also demonstrated bony erosion and the presence of hyperdense material within some of the sinuses (Fig. 6-17). Aviv et al<sup>69</sup> reported a case of *Exserohilum* pansinusitis with multiple intracranial mucocoeles. CT showed expansile bilateral sinus and nasal masses. The lesions had extended laterally into the orbits and the floor of the anterior cranial fossa. The lesions showed heterogeneous density with some areas of hyperdensity and others of hypodensity on CT. On MR the maxillary sinus and sphenoid were hyperintense on T2WI, indicating a high water content of obstructed secretions. But both ethmoids showed hypointense signal, representing areas of fungi and dried secretions. These areas of hypointensity could be confused with air on the T2WI. The extent of sinus opacification could more easily be appreciated on the CT scan.

CNS phaeohyphomycosis has been reported secondary to *Bipolaris* (Fig. 6-18), *Chaetomium*,<sup>70</sup> *Curvularia*,<sup>71,72</sup> *Fonsecaea*,<sup>71</sup> *Wangiella*,<sup>72</sup> and *Cladophialophora*.<sup>78</sup> *Cladophialophora*, a highly neurotropic fungus, has been the etiologic agent in approximately half of reported dematiaceous fungal brain abscesses.<sup>73</sup> Non-enhancing low-density lesions on CT have been described with CNS *Curvularia*.<sup>74</sup> Multiple enhancing lesions have also been reported with *Curvularia*.<sup>75</sup> Shields et al<sup>76</sup>



**Figure 6-18** Phaeohyphomycosis: brain infarcts caused by *Bipolaris*. Non-enhanced axial CT scan at the level of the frontal horns shows a large hemorrhagic infarct (asterisk) in the right frontal lobe and multiple bland infarcts involving both cerebral hemispheres (arrows).

reported that *Cladophialophora* presented as multiple areas of high T2 signal in the thoracic spinal cord and conus medullaris in a woman with pulmonary sarcoidosis on oral steroids. The diagnosis was made following spinal cord biopsy as the patient progressed on steroids. Shimosa and Waga<sup>77</sup> reported a case of cerebral phaeohyphomycosis granuloma (unspecified genus) that was complicated by meningitis. Multiple mycotic aneurysms formed after resection of the granuloma followed by a second hemorrhage. Arteriography demonstrated four mycotic aneurysms involving the middle and anterior cerebral arteries. The mortality is 100% without treatment and high (65%) despite the combination of surgical and antifungal treatment.<sup>78</sup>

## Pseudallescheriasis

Sinus and CNS involvement by *Pseudallescheria* (*Scedosporium apiospermum*) is rare.<sup>79</sup> Most reports of *Pseudallescheria* sinusitis have involved the maxillary sinuses, with a few instances of ethmoid, frontal, and sphenoid sinus involvement.<sup>87,103-105</sup> Gluckman et al<sup>80</sup> reported a case of a diabetic man with *Pseudallescheria* sinusitis involving the maxillary sinus. CT showed erosion of the right orbit medial wall. Orbital apex syndrome secondary to a sphenoidal sinus mycetoma of *Pseudallescheria boydii* has also been reported.<sup>81</sup> Infection can present as diskospondylitis and rarely as calvarial osteomyelitis.<sup>82</sup> Intracranial infection is usually by direct or hematogenous extension, including cerebral abscesses, meningitis, cerebritis, ventriculitis, and intracranial vascular involvement.<sup>83</sup> On CT, the *Pseudallescheria* brain abscesses showed ring enhancement.

Most patients who contract a CNS infection with *Pseudallescheria* are either immunocompromised or are victims of near drowning.<sup>84</sup> In this report, with brain abscesses secondary to *Pseudallescheria*, four of the patients had suffered near-drowning episodes. Although initial CT scans were normal, CT scans performed 2–4 weeks after the near-drowning episodes revealed multiple ring-enhancing brain abscesses.

Kershaw et al<sup>85</sup> reported a case of *Pseudallescheria* infection that resulted in a series of cerebral infarcts. The initial CT scan revealed a low-density lesion of the thalamus. Arteriography showed occlusion of the right posterior cerebral artery. Autopsy disclosed necrosis and thrombosis of multiple vessels. Selby<sup>86</sup> reported a case of pachymeningitis secondary to *Pseudallescheria* that produced progressive paraplegia. Myelography demonstrated an extradural lesion from T6 to T10 with a block at T9.

## Sporotrichosis

*Sporothrix schenckii* typically produces cutaneous lesions and rarely affects the CNS. There have been several case reports of chronic sporotrichosis meningitis.<sup>93,94</sup> Agger et al<sup>87</sup> reported a case of ocular sporotrichosis in a diabetic with necrotizing ethmoid sinusitis. Radiographs demonstrated opacification of the ethmoid air cells with erosion of the lateral wall. Kumar et al<sup>88</sup> described a case of sinonasal sporotrichosis with intracranial extension.

## Zygomycosis

Zygomycoses are classified as either mucormycoses or entomophthoromycoses. Mucormycosis usually manifests in immunocompromised individuals as an acute, necrotic, rapidly progressive disease that may lead quickly to death. Entomophthoromycosis is a chronic, slowly progressive subcutaneous disease seen mostly in immunocompetent patients living in tropical climates.<sup>89</sup> Cerebral mucormycosis occurs in three different ways:

- rhinocerebral, usually secondary to infiltration from a primary site within sinuses
- systemic with hematogenous spread to the CNS typically from the lungs
- isolated cerebral form with the no other body sites involved.

The isolated form is rare and may present as a single or multiple parenchymal lesions or meningoencephalitis. It usually occurs in intravenous drug abusers and has been described with penetrating brain injury, and in patients with AIDS and non-Hodgkins lymphoma.<sup>90</sup> The chronic rhinocerebral form of mucormycosis can sometimes occur, usually in patients with diabetes and ketoacidosis. Skull base osteomyelitis can be seen in these patients, unlike in the acute form in which the bone involvement is uncommon.<sup>91,92</sup> Chan et al reported a case with extensive skull base osteomyelitis due to chronic isolated sphenoid sinus disease.<sup>92</sup> Rhino-orbital-cerebral zygomycosis is the most common manifestation of zygomycosis.<sup>93</sup> Cerebral and sinoorbital zygomycosis can occur independently. Sinoorbital infection usually begins in the nasal cavity and spreads contiguously to the adjacent paranasal sinuses and orbit. Isolated cerebral zygomycosis usually has a hematogenous origin. Vascular invasion and subsequent thrombosis are common in zygomycosis.

Gamba et al<sup>94</sup> reported that early paranasal sinus involvement appeared as mucosal thickening on CT scans usually without air–fluid levels. *Bone destruction was unusual and seen late even with spread beyond the paranasal sinuses.* Air–fluid levels in the sinus, calcified sinus mass, thickening and erosion of the sinus wall were also described.<sup>103,104</sup> Press



et al<sup>95</sup> reported sinus involvement as hyperintense secretions and mucosal thickening on T2WI. Administration of paramagnetic contrast typically shows enhancement of the infected mucosa. If vascular thrombosis has occurred, it can result in paradoxical non-enhancement of the diseased tissues.

Extension into the pterygopalatine fossa and infratemporal fossa can occur without bone destruction. Gamba et al<sup>94</sup> reported deep tissue involvement in seven of 10 patients with zygomycosis, but bone destruction in two. Loss of the normal fat density and obliteration of fat tissue planes on CT were indicative of deep tissue involvement. Press et al<sup>95</sup> reported swollen muscles with increased signal on T2WI and PD images in the parapharyngeal and infratemporal regions.

Orbital spread from the paranasal sinuses may occur by direct extension through the thin lamina papyracea or ethmoidal blood vessels.<sup>96</sup> *Radiographic signs of orbital involvement include proptosis, preseptal edema, infiltration of the orbital fat, thickened optic nerve, thickened extraocular muscles, lateral displacement of the medial rectus, extraconal abscess, and non-enhancement of ophthalmic artery or vein.*<sup>94,97,95</sup> Infection can advance into the cavernous sinus through the ophthalmic artery or other orbital vessels.<sup>96</sup> Cavernous sinus involvement can be suspected when imaging demonstrates bulging of the lateral wall of the sinus. Non-enhancement of the cavernous sinus on CT or MR is indicative of thrombosis (Fig. 6-19).

The CNS involvement can result in meningitis, cerebritis, abscess, and infarction.<sup>94,95</sup> The basal frontal lobes and temporal lobes are most frequently involved when the cerebral infection is from direct extension of rhino-orbital zygomycosis. Gamba et al<sup>94</sup> reported cerebral abscesses as low-density masses on CT with variable peripheral enhancement and little surrounding vasogenic edema. Berthier et al<sup>98</sup> reported a case with initial CT showing a small nodular enhancing lesion surrounded by edema in the frontal lobe. After a good response to amphotericin B the patient became more symptomatic one month later. CT done at this time revealed a large multiloculated ring-enhancing frontal lobe abscess.

On MR, cerebral involvement will show areas of abnormal increased signal on T2WI and FLAIR images.<sup>95</sup> Areas of cerebritis can enhance after administration of paramagnetic

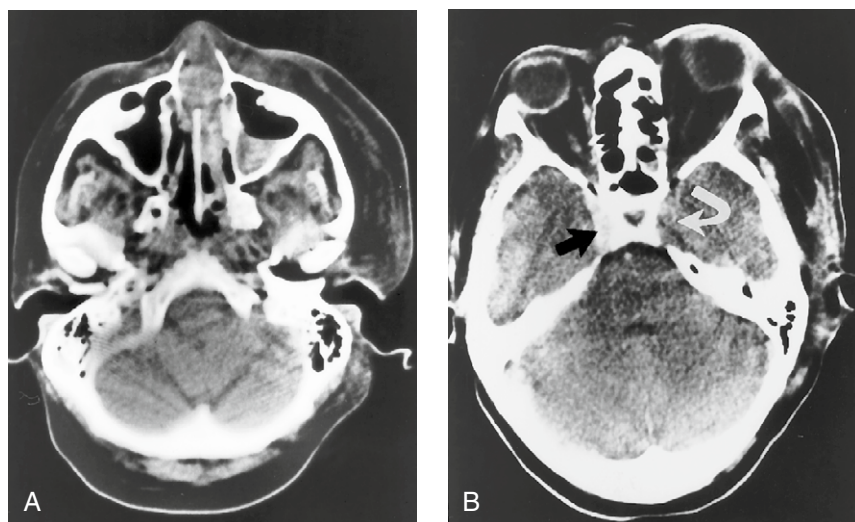
contrast. Vascular invasion frequently leads to thrombosis and subsequent cerebral infarction. On CT, infarcts will appear as areas of low density in a vascular distribution. On MR the infarcts will have decreased T1 and increased T2 signal. There can be enhancement, and hemorrhage can develop in areas of infarction. Vascular invasion can also result in septic emboli, which can lead to foci of cerebritis distant from sinoorbital infection. Diffusion restriction seen as a bright signal may be due to either acute infarctions or cerebritis.<sup>99</sup>

Escobar and Del Brutto<sup>100</sup> described an immunosuppressed patient with multiple brain abscesses. Zygomycosis has a predilection for vascular invasion, particularly the internal carotid artery (Fig. 6-20). Courey et al<sup>101</sup> reviewed the angiographic findings of craniofacial zygomycosis and described arteritis, stenosis, occlusion, pseudoaneurysm formation, embolism, and infarction. On MR the presence of signal in an artery instead of the expected flow void indicates occlusion or very slow flow. MR/CT angiography can be used to evaluate vessel patency, if patients are able to cooperate during the study.

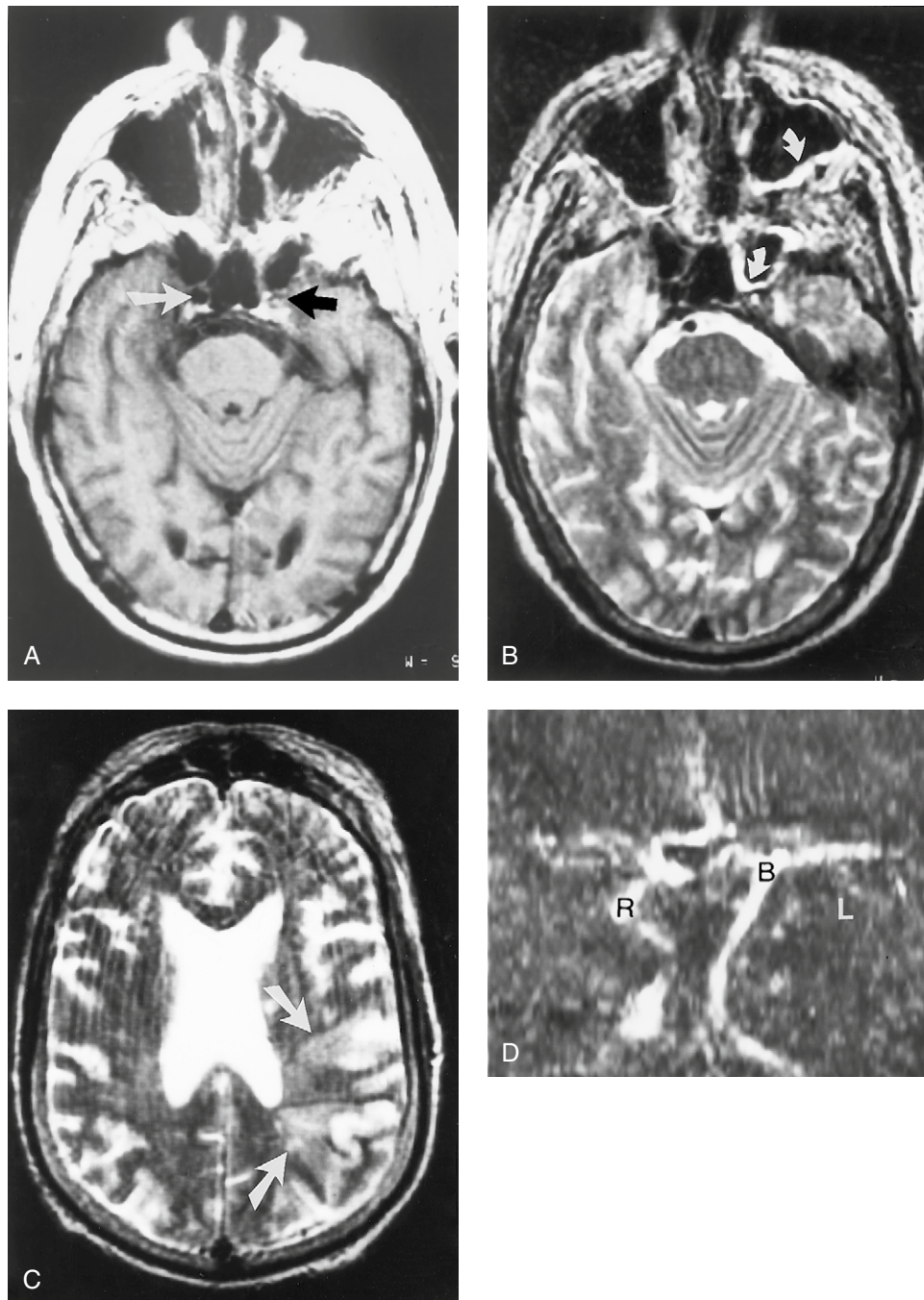
## Thoracic mycotic disease

### Aspergillosis

*Aspergillus* organisms produce five basic forms of thoracic infection in humans: allergic bronchopulmonary, saprophytic (aspergilloma), chronic necrotizing (semi-invasive), airway invasive, and angioinvasive aspergillosis. The thoracic manifestations of aspergillosis are related to the number and virulence of these fungi, immune state of the individual and the presence and extension of underlying lung disease.<sup>102,103</sup> The allergic form is seen predominantly in atopic individuals who demonstrate hyperreactivity to the fungus. The saprophytic form represents a non-invasive colonization of a preexisting pulmonary cavity by *Aspergillus* in an immunocompetent host leading to a mycetoma (aspergilloma). In mildly immunosuppressed individuals, *Aspergillus* may develop a more aggressive localized form of infection (chronic necrotizing aspergillosis) in which the fungus creates an inflammatory



**Figure 6-19** Rhinocerebral zygomycosis with cavernous sinus thrombosis. (A) Axial CT scan through the maxillary sinuses shows an air–fluid level in the left maxillary sinus and swelling of the left facial soft tissues. (B) Enhanced CT scan shows normal enhancement of the right cavernous sinus (black arrow) and no enhancement of the thrombosed left cavernous sinus (white curved arrow).



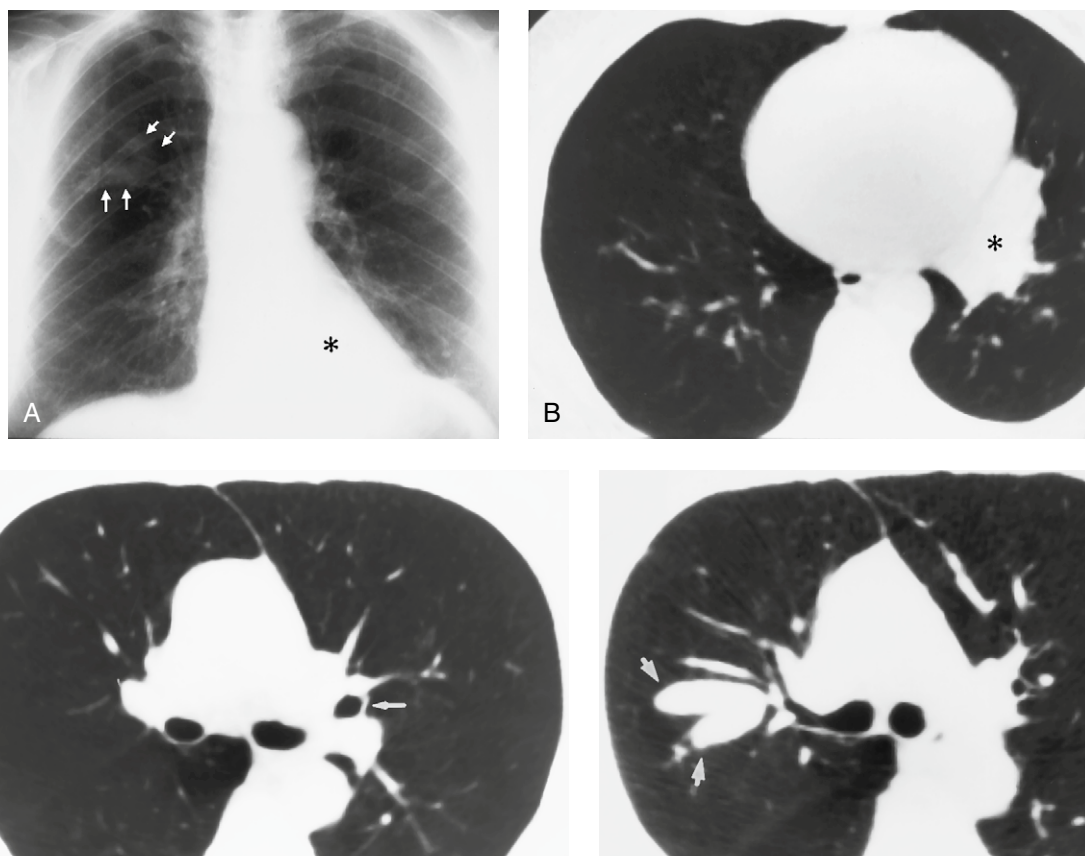
**Figure 6-20** Rhinocerebral zygomycosis with occluded internal carotid artery and multiple infarcts. (A) Axial T1-weighted image shows normal signal void of the right internal carotid artery (white arrow) and abnormal increased signal within the occluded left internal carotid artery (black arrow). (B) Axial T2-weighted images shows hyperintense mucosal thickening of the left maxillary and sphenoid sinuses (small white curved arrows). (C) Axial T2-weighted image shows wedge shaped hyperintense infarcts in the left posterior temporal-parietal region (white arrows). (D) Frontal view from a 3D time of flight MR angiogram shows normal flow, bright signal, in the right internal carotid artery (black "R") and basilar artery (black "B"). No flow is detected in the expected location of the left internal carotid artery (white "L").

process within normal lung parenchyma that results in tissue necrosis, cavitation, and formation of an aspergilloma.<sup>104</sup> *In the invasive form, the fungus produces a granulomatous bronchopneumonia that typically affects severely granulocytopenic individuals.* On occasion, one form of disease will transition into another and some authors believe this is a spectrum of one disease.<sup>105</sup>

#### Allergic bronchopulmonary aspergillosis

Allergic bronchopulmonary aspergillosis (ABPA) is characterized by the presence of inspissated mucus plugs containing *Aspergillus* and inflammatory cells, especially eosinophils. Besides mucoid impaction of bronchi, bronchocentric

granulomatosis (necrotizing granulomas centered upon the airway lumens), eosinophilic pneumonia, and chronic or exudative bronchiolitis are also seen.<sup>106</sup> McCarthy et al<sup>107</sup> reported the chest radiographic features in 111 patients with ABPA. Radiographic findings are predominantly related to acute and chronic inflammation within the pulmonary parenchyma and airways. Infiltrates range in size from lobar airspace consolidation to subsegmental patchy opacities. The airspace opacity may be from an eosinophilic pneumonia or by postobstruction pneumonitis or atelectasis (Fig. 6-21). More than one infiltrate may be present at a time, and may be bilateral. Although upper lobes are most frequently affected, airspace disease occurs in all lobes.<sup>107</sup>



**Figure 6-21** Allergic bronchopulmonary aspergillosis. (A) Posteroanterior (PA) chest radiograph of a 65-year-old woman reveals abnormal opacity in the left retrocardiac area (asterisk) which causes non-visualization of the medial portion of the left hemidiaphragm and the retrocardiac descending aorta. Note the subtle “v-shaped” opacity within the right upper lobe (arrows). (B) Chest CT image (lung window) through the lower thorax shows that the retrocardiac opacity in (A) represents airless consolidation within the anteromedial basal segment of the left lower lobe (asterisk). (C) Chest CT image (lung window) reveals central bronchiectasis involving apical posterior bronchus in left upper lobe (arrow). (D) Chest CT image (lung window) demonstrates a “v-shaped” opacity (arrows) representing impacted mucus within ectatic bronchi in the posterior segment of the right upper lobe, corresponding to that seen in (A).

The radiographic features produced by ABPA have been described as “tram-line shadow,” “gloved-finger shadow,” etc. These refer to opacities created by airway inflammation, with or without mucoid filling of the affected bronchus. “Tram-lines” are subtle, parallel hairline opacities representing a thick-walled bronchus. Most tram-line opacities were identified in patients less than 15 years of age.<sup>107</sup> Tram-line opacities tend to be transient. *Central bronchiectasis is one of the most characteristic findings of ABPA* (see Fig. 6-21). “Parallel-line shadows” represent walls of dilated bronchi. In patients with ABPA, these lines are common, often bilateral, and more frequent in the upper lobes. Mucoid impaction of the bronchi creates the most characteristic “toothpaste shadows,” which are short oblong opacities 5–8 mm wide, representing mucoid impaction within dilated bronchi (see Fig. 6-21). “Gloved-finger shadow” is a band-like opacity with an expanded, rounded distal end, representing secretions in a dilated bronchus with an occluded distal end (Fig. 6-22). Occasionally, an air–fluid level is identified within the lumen of such a bronchus, whereas in other patients the bronchus may be filled with mucoid material, creating a 1–2 cm spherical

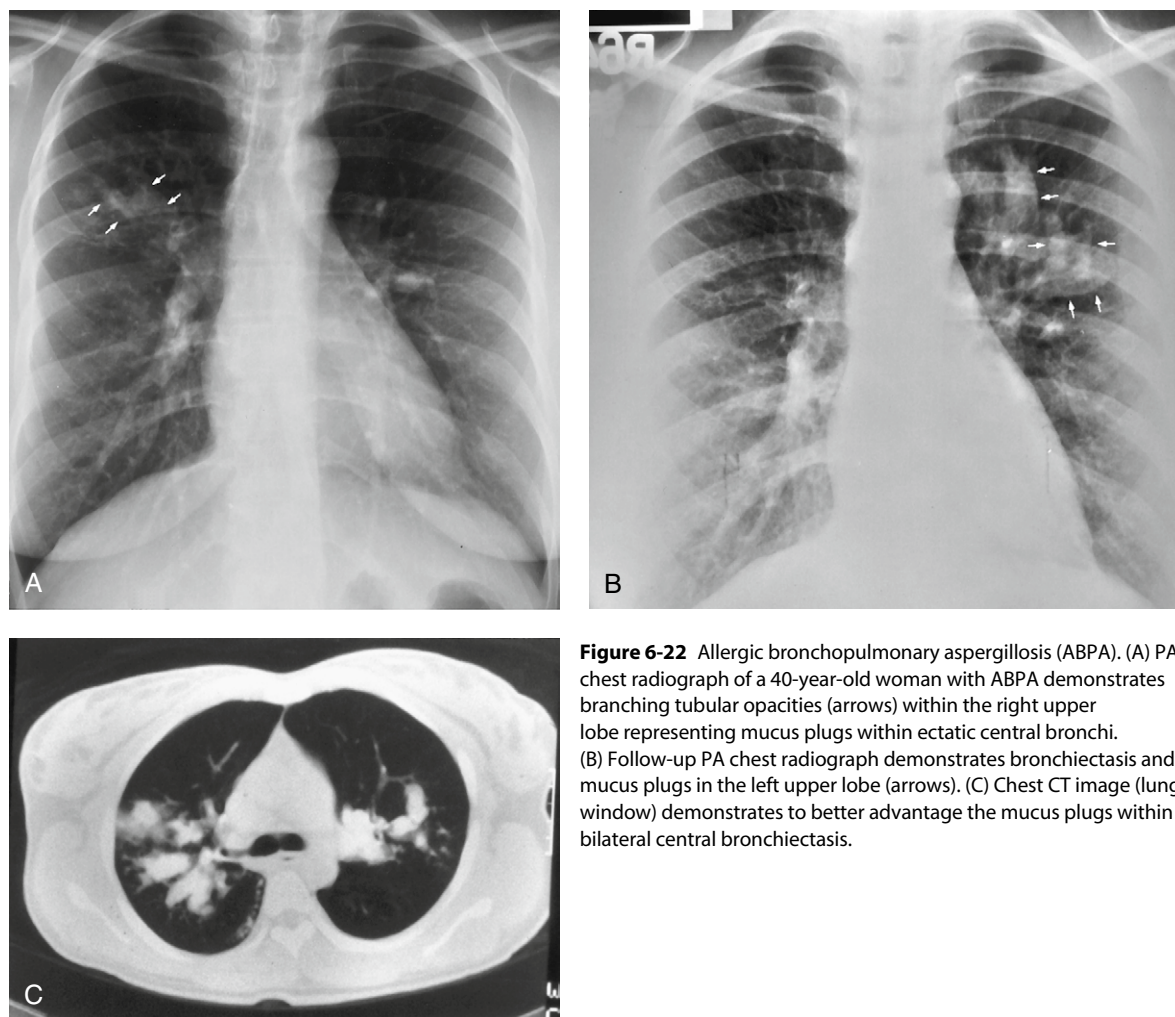
mass (see Fig. 6-22). Bronchiectasis, most often found in the upper lobes, has been reported in 25–65% of patients. On CT, ABPA imaging findings consist primarily of mucoid impaction and bronchiectasis involving predominantly the upper lobes or medial two-thirds of the lungs.<sup>108</sup>

#### Saprophytic aspergillosis (Aspergilloma)

The typical aspergilloma is a saprophytic non-invasive fungal pulmonary infection. Aspergilloma is a specific type of pulmonary mycetoma or fungus ball, albeit the most common. It occurs within preexisting lung cavities, in portions most severely damaged by the fibrocavitary and fibrocystic lesions, the upper or the superior segments of the lower lobes. Aspergilloma have been reported in lung abscess, bronchogenic cyst, emphysema, radiation fibrosis, and cavitary lung carcinoma, etc.<sup>104</sup> Interestingly, aspergilloma also occur in cavities created by other fungal infections.

The classic aspergilloma manifests on chest roentgenograms as a homogeneous, rounded, mobile opacity representing a tangled mass of *Aspergillus* hyphae, fibrin, mucus and cellular debris within a spherical or ovoid upper





**Figure 6-22** Allergic bronchopulmonary aspergillosis (ABPA). (A) PA chest radiograph of a 40-year-old woman with ABPA demonstrates branching tubular opacities (arrows) within the right upper lobe representing mucus plugs within ectatic central bronchi. (B) Follow-up PA chest radiograph demonstrates bronchiectasis and mucus plugs in the left upper lobe (arrows). (C) Chest CT image (lung window) demonstrates to better advantage the mucus plugs within bilateral central bronchiectasis.

lobe cavity (Fig. 6-23). Occasionally, an individual may have multiple aspergilloma (Fig. 6-24). A *second characteristic feature is a narrow crescent-shaped lucency or air crescent sign that separates the aspergilloma from the non-dependent wall of the cavity*. Occasionally the aspergilloma fills the cavity so that the air crescent sign may be absent or easily overlooked (see Fig. 6-24). As the patient changes position from upright to recumbent to lateral decubitus, the mycetoma moves to the gravitationally dependent portion of the cavity (Fig. 6-25). The portion of the wall nearest the pulmonary hilum is thin, and the wall adjacent to the pleura is typically much thicker (see Figs 6-23, 6-24). Although the *Aspergillus* organism does not invade the wall, it does induce an acute and chronic inflammatory reaction within the adjacent pleura. New pleural thickening adjacent to chronic cavitary or cystic lung disease may herald the formation of an aspergilloma.<sup>109</sup>

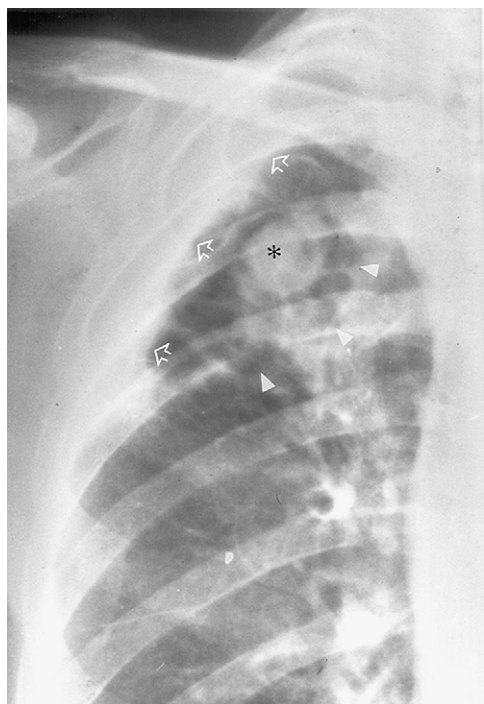
The aspergilloma is frequently not obvious on chest radiographs due to distortion caused by fibrosis. CT is more sensitive than plain radiography in identifying an aspergilloma. CT may show air lucencies within the mass, creating a spongeliike appearance (Fig. 6-26). Less commonly, only fronds of opacity project into the cyst lumen from its wall. Other atypical features include a fluid level and, rarely, calcifications within the

aspergilloma. Spontaneous resolution has been documented in 7–10% of cases.

Although aspergillomas are not invasive, they may be the source of subsequent *Aspergillus* dissemination.<sup>104</sup>

#### Chronic necrotizing aspergillosis (Semi-invasive aspergillosis)

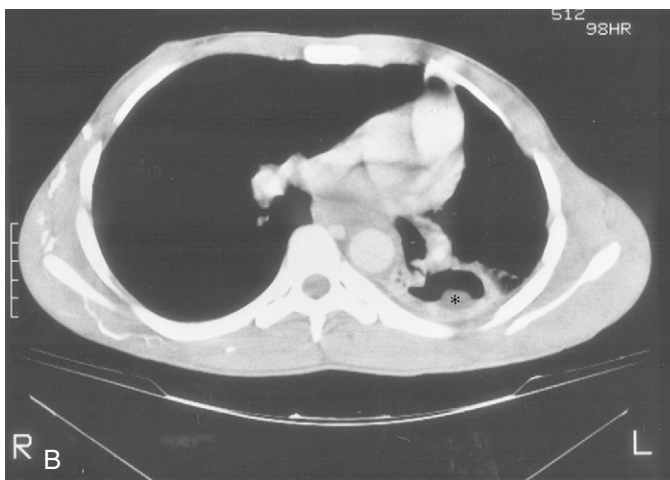
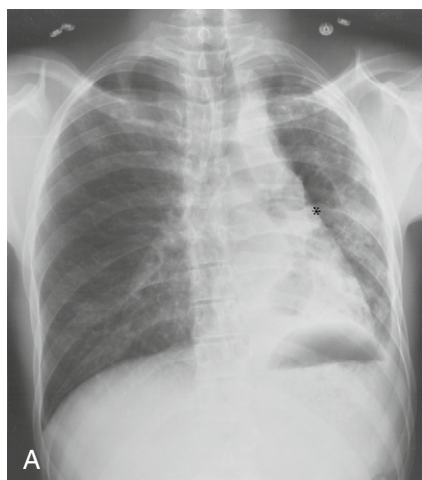
In patients who are mildly immunocompromised (alcoholism, diabetes mellitus, etc.) or who have lung disease (sarcoidosis, radiation fibrosis, chronic obstructive pulmonary disease), *Aspergillus* may create a chronic, localized infection that is more aggressive than the classic non-invasive aspergilloma but significantly more protracted than invasive pulmonary aspergillosis.<sup>109,110</sup> Originally termed semi-invasive aspergillosis,<sup>103</sup> it is more commonly referred to as chronic necrotizing aspergillosis (CNA). In CNA the *Aspergillus* organism produces a chronic inflammatory process with limited tissue invasion, with tissue necrosis, granulomatous inflammation and cavity formation.<sup>104</sup> At the same time, an aspergilloma often develops within the necrotic tissue as a secondary phenomenon.<sup>104</sup> Most patients with CNA are middle aged and have underlying non-cavitary pulmonary disease or are mildly immunocompromised.<sup>110</sup>



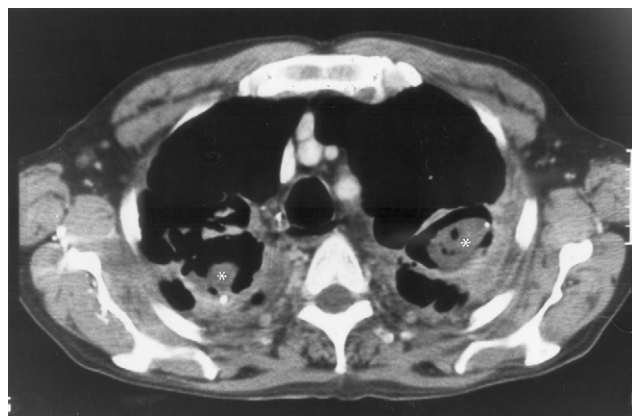
**Figure 6-23** Aspergilloma. Coned anteroposterior (AP) chest radiograph of a recumbent 53-year-old man demonstrates a rounded mass (*asterisk*) in the apex of the right lung with surrounding lucency representing cystic lung disease. The wall of the cystic area is very thin along its caudal aspect toward the right hilum (*arrowheads*), while the wall of the cystic disease and the adjacent pleura is markedly thickened along its apical and lateral surface (*open arrows*).



**Figure 6-24** Aspergillomas in an adult with stage IV sarcoidosis. PA chest radiograph shows bilateral aspergillomas (*asterisks*) within the upper lobes creating opacities which vary in size. The severe lung disease tends to obscure the smaller aspergilloma and their associated findings. Note the variation in the width of the pleural thickening (*arrows*) adjacent to the aspergilloma and the subtle air crescents separating aspergilloma from pleural thickening.



**Figure 6-25** Aspergilloma in a 24-year-old man with previous tuberculosis, a 2-day history of productive blood-tinged sputum, and a weight loss of 17 pounds. (A) Upright PA chest radiograph shows extensive volume loss and marked bronchiectasis in the left lung with surrounding consolidation and small nodular opacities. A rounded mass (*asterisk*) is present in the dependent portion of one of the bronchiectatic areas. The air crescent surrounding the mass is quite large. Fluid obscures the dependent portion of the mass. Both features make the correct diagnosis, aspergilloma, difficult. (B) Contrast-enhanced chest CT image (soft tissue window) with the patient in the supine position shows that the mass (*asterisk*) has moved to the dependent portion of the "cystic" lesion. Note the wide air crescent and the small amount of fluid adjacent to the dependent portion of the mass. Left pneumonectomy confirmed an aspergilloma and extensive bronchiectasis.



**Figure 6-26** Aspergillomas in a 52-year-old man with hemoptysis. Chest CT image (soft tissue window) reveals a single, rounded, low-attenuation mass (asterisks) within the dependent portion of a cyst within the apical portion of each upper lobe. The mass on the left shows internal air lucencies. The cyst walls are thickened along the portion contacting pleura. Surgery proved that both masses were aspergilloma.

Semi-invasive aspergillosis usually presents as upper lobe consolidation that progresses to cavitation, with multiple areas of nodular opacity, which may be associated with pleural thickening.<sup>108</sup> This radiographic appearance may be indistinguishable from that of pulmonary tuberculosis.<sup>111</sup> The radiographic features of CNA may also be similar to those of non-invasive aspergilloma: a mobile, homogeneous rounded opacity with an adjacent air crescent lying within an upper lobe cavity that exhibits a thin wall toward the lung hilum and a thickened wall along its pleural margin. However, there is one important difference. As originally described,<sup>103</sup> the aspergilloma of CNA develops within lung parenchyma that had no previous cyst or cavity. The diagnosis of CNA is suggested when a mycetoma is present in lung without previous cystic or cavitory disease. Rarely, CNA is imaged as it progresses from a localized area of chronic alveolar infiltrate to cavitation and subsequent mycetoma formation within the cavity. The progression of disease varies widely from several weeks to many years.<sup>104</sup>

Sider and Davis<sup>112</sup> described three patients with a localized form of aspergillosis in which the disease manifested as rounded nodules or masses of 1, 2, and 10 cm diameter. None of these patients was severely immunocompromised, receiving prolonged therapy (corticosteroids, antibiotics or chemotherapy), or had preexistent pulmonary disease or an abnormal chest radiograph. Multiple cavitory nodules more than 1 cm in diameter have also been reported as a common pattern of semi-invasive pulmonary aspergillosis in patients with chronic obstructive pulmonary disease (COPD).<sup>113</sup>

#### Invasive pulmonary aspergillosis

Immune-suppressed patients, particularly those with leukemia or lymphoma and chemotherapy-induced neutropenia, patients with organ transplants, and patients receiving high doses of corticosteroids or broad-spectrum antibiotics are prone to contracting invasive pulmonary aspergillosis (IPA).<sup>112</sup> In most patients the lungs are affected, and infection remains confined. Thoracic disease can manifest as necrotizing bronchopneumonia, hemorrhagic infarction, and tracheobronchitis.

In susceptible hosts, *Aspergillus* colonizes the tracheobronchial tree and invades the walls of large bronchi, resulting in extensive ulceration and sloughing. When infection is confined to the airway, chest radiographs are normal or only show foci of atelectasis, even though patients might be quite symptomatic. *Aspergillus* pneumonia develops when the organism invades lung parenchyma or, more commonly, when the infection centers on terminal airways and extends into the adjacent lung. *It is important to be aware that a patient's chest radiographs may appear normal in up to 25% of cases of IPA pneumonia*<sup>114</sup> or demonstrate non-specific, patchy foci of airspace consolidation. With extension of the infection into lung, the organism typically invades the adjacent pulmonary artery, causing thrombosis. Radiographically, these foci of bronchopneumonia may be easily overlooked. With time, the consolidations grow, and chest radiographs reveal one or more mass-like opacities several centimeters in diameter that are often peripheral in location (Fig. 6-27). On CT images, the most common appearance is a rounded mass, representing necrotic lung infiltrated with *Aspergillus* hyphae,<sup>152,169,170</sup> which are surrounded and separated from normal lung by a thin zone of ground-glass opacity that is of lower attenuation than the central mass but of higher attenuation than the surrounding normal lung.<sup>117,122,123</sup> Kuhlman and associates<sup>114</sup> termed this characteristic appearance the "CT halo sign" (see Fig. 6-27), proven in CT-pathologic correlations to represent hemorrhage.<sup>162,171</sup> Although not specific for IPA, this sign is most often secondary to IPA. *The halo sign appears early in the course of infection, often preceding the development of cavitation or air crescent by 2 to 3 weeks.*<sup>114</sup> In leukemic patients with chemotherapy-induced neutropenia, these rounded infiltrates on CT and chest radiographs are so characteristic of IPA that some consider them virtually diagnostic.<sup>128</sup> CT may also demonstrate multiple, small, irregular inflammatory masses that may coalesce into clusters of small, fluffy nodules, and large areas of consolidation.<sup>115</sup>

Herold and associates<sup>247</sup> examined 37 nodular IPA lesions in 11 patients by MRI. On T1WI/T2WI or both, 34 of the 37 lesions demonstrated a hypointense or isointense center and a rim of higher signal intensity, referred to as a target appearance. In five lesions, the target appearance was caused by central cavitation. In all others, they believed the center represented coagulative fungal necrosis. The higher signal intensity in the periphery of each lesion corresponded to subacute hemorrhage or hemorrhagic infarction. All lesions demonstrated peripheral enhancement with gadolinium diethylene-triamine pentaacetic acid (Gd-DTPA), believed to be from inflammation. In seven of 11 lobar consolidations the lesions were hyperintense, compatible with hemorrhagic infarction or hemorrhage alone. The authors concluded that the "target" appearance on MRI will prove to be another diagnostic key in early IPA.

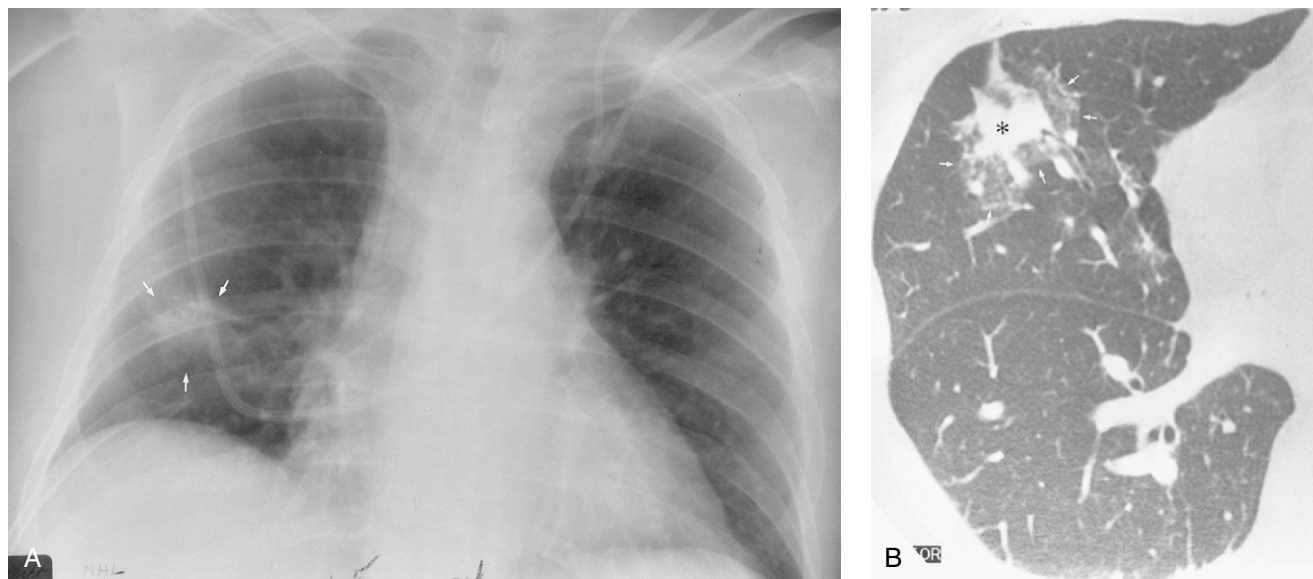
Cavitation occurs in up to half of patients with infiltrates due to IPA, and approximately 80% of cavities demonstrate air crescents.<sup>116</sup> Air crescent formation is characteristic of the recovery phase of IPA, and the presence of granulocytes is crucial for the formation of pulmonary cavitation and typically occurs 3–4 days after WBC counts reach a level of 1000/mm<sup>3</sup> or greater.<sup>116</sup> Although the radiographic appearance is identical to saprophytic aspergilloma in chronic cystic lung disease, the two processes must not be confused. During air crescent formation in IPA, resorption of necrotic, hemorrhagic tissue



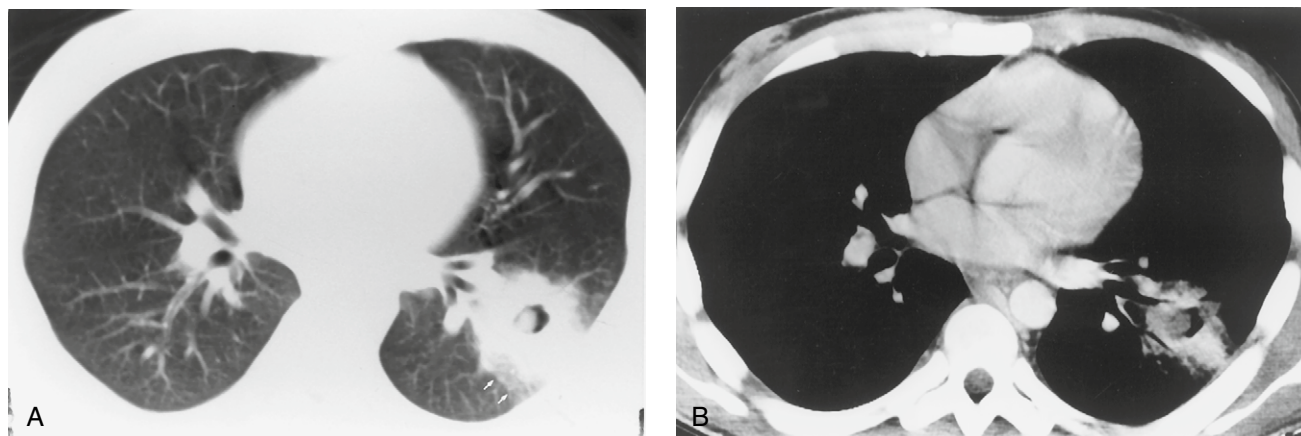
at the periphery of the infiltrate leads to a central sequestrum of non-viable pulmonary tissue infiltrated with *Aspergillus* hyphae and surrounded by air (Fig. 6-28).<sup>126,145</sup> Although characteristic of IPA, air crescents are of relatively little diagnostic use, because they occur relatively late in the course of the pneumonia. With healing, pulmonary opacities become smaller and often more well defined. Many will resolve. However, with the development of cavitation, approximately 25–50% of patients experience massive hemoptysis, typically for 1–2 days after the appearance of the air crescent.<sup>153,154</sup> Occasionally, the mass of necrotic lung surrounded by the air crescent does not totally resolve and may become a source of

infection should the patient receive another cycle of chemotherapy.

Although regression of *Aspergillus* pneumonia does not guarantee recovery, failure of the infiltrates to cavitate and show regression is a harbinger of a fatal outcome in most patients.<sup>116</sup> In such cases one or more foci enlarge, new infiltrates develop, or both are seen. Less commonly a wedge-shaped or triangular opacity that abuts a pleural surface may develop, representing hemorrhagic infarction or bronchopneumonia.<sup>117,114</sup> Clinically patients often experience chest pain or hemoptysis with the appearance of this pattern of infiltrate, or both.<sup>117</sup> Despite radiographic progression of the infection, IPA does not



**Figure 6-27** Invasive pulmonary aspergillosis in a 47-year-old man with low-grade stage IV non-Hodgkin lymphoma treated with bone marrow transplantation and chemotherapy. (A) AP bedside chest radiograph demonstrates focal, poorly margined airspace opacity (arrows) in the right mid lung. A central venous catheter is also present. (B) High-resolution chest CT image (lung window) shows an irregular, 2 cm nodular opacity (asterisk) in the right upper lobe, surrounded by ground-glass attenuation, “halo” sign (arrows). Autopsy revealed invasive pulmonary aspergillosis with multiorgan dissemination.



**Figure 6-28** Invasive pulmonary aspergillosis in a 27-year-old man with end-stage acute myelogenous leukemia. (A) Chest CT image (lung window) shows an ill-defined opacity with central cavitation containing a small mass. Note the “halo” sign of ground-glass attenuation (arrows) adjacent to the posterior aspect of the opacity. (B) Chest CT image (soft tissue window) through the same opacity 2 weeks later demonstrates the cavitary opacity with an intracavitary low attenuation mass representing devitalized lung infiltrated with *Aspergillus* hyphae. IPA was diagnosed by bronchoscopy. The patient died despite amphotericin B therapy.



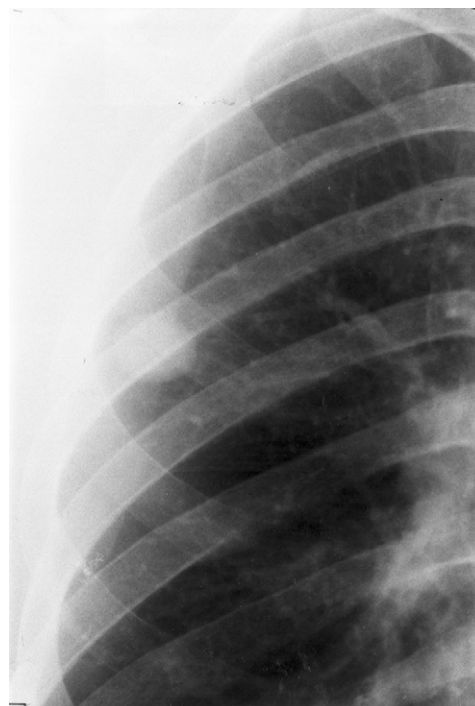
**Figure 6-29** Mass-like consolidation in blastomycosis. PA chest radiograph shows a large, mass-like consolidation with central air bronchograms in the right upper lobe. Multiple, subtle patchy focal opacities (arrows) are present in both the right and left lungs (satellite lesions). Pleura located superolaterally to the consolidation demonstrates subtle thickening.

frequently extend into the pleural space. Pleural aspergillosis is more frequently a complication of thoracic surgery or the result of rupture of a mycetoma cavity into the pleural space.<sup>118</sup> Uncommonly, IPA may create miliary and reticulonodular patterns and represent hematogenous pulmonary seeding.<sup>117</sup>

## Blastomycosis

In a retrospective review of 27 cases of pulmonary blastomycosis, Halvorsen et al<sup>119</sup> described four radiographic patterns of disease: airspace disease, nodular masses, interstitial disease, and cavitation. Furthermore, they found no relationship between the radiographic pattern and distribution, pulmonary symptoms, or clinical stage. Most authors indicate that airspace consolidation is the most common radiographic appearance of pulmonary parenchymal involvement with blastomycosis. However, in a series of 33 patients,<sup>120</sup> consolidation was the second most frequently observed manifestation, with a prevalence of 26–76%.<sup>119–122</sup>

The pulmonary disease manifests as a homogeneous or patchy alveolar opacity, varying from a rounded, ill-defined, solitary mass-like opacity to multiple nodular opacities (Fig. 6-29).<sup>162,167</sup> In another study (63 patients) consolidation, either segmental or non-segmental, was present in 59% and in a minority (19%) lobar distribution was seen.<sup>123</sup> Although airspace opacities typically involve less than a lobe, they are usually large. Most pulmonary consolidations have ill-defined margins and are peripheral or abut a fissure. Within regions of consolidation, air bronchograms are commonly present on radiographs. In one series, the upper lobes are more frequently involved.<sup>124</sup> The next most common radiographic manifestation is single or multiple pulmonary masses (Fig. 6-30). The prevalences vary from 4% to 31%.<sup>120,163,166</sup> In the Mayo Clinic series,<sup>120</sup> a pulmonary mass was the most common manifestation. Masses are usually quite large, ranging from 4 to 10 cm in diameter (mean 6 cm),<sup>120</sup> and most have shaggy or irregular margins (Fig. 6-31). They are typically indistinguishable from lung carcinoma, and in one series<sup>125</sup> 55% were



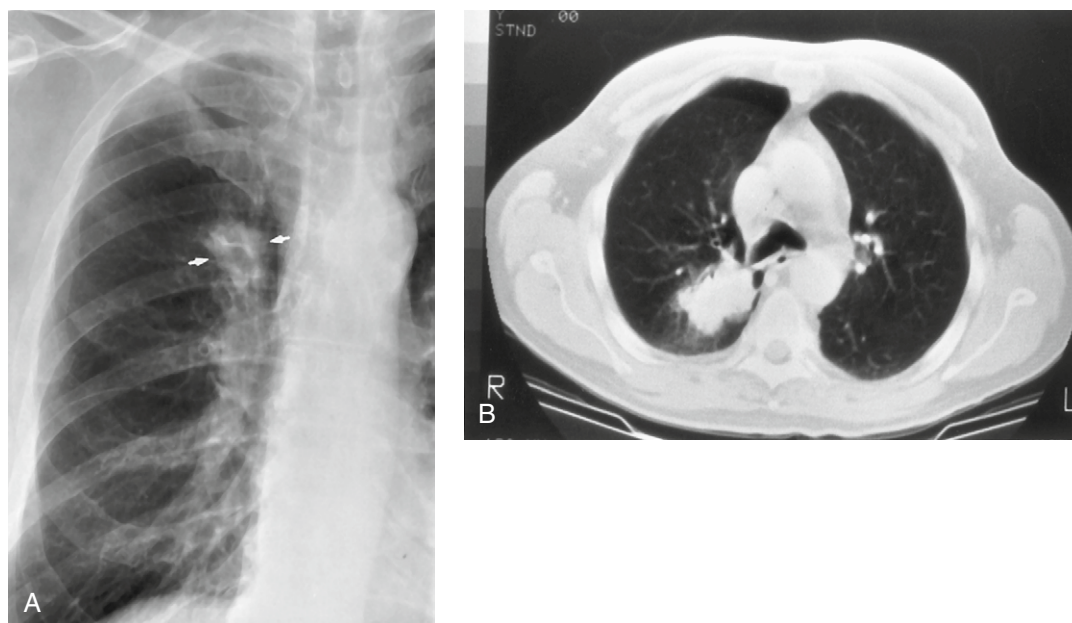
**Figure 6-30** Peripheral lung mass blastomycosis. PA chest radiograph coned to upper right lung demonstrates a sharply margined, homogeneous, spherical mass in the lung periphery. There are no calcifications or cavitation.

diagnosed by surgery. *Air bronchograms occur uncommonly and cavitation is rare.* Disease occurs in the upper lobes and lower lobes with equal frequency.<sup>120</sup> Masses are often central (see Fig. 6-31) or paramediastinal<sup>138,143</sup> (see Fig. 6-30). In a CT study, masses extended to the hilum in 86% of patients.<sup>124</sup> Rarely, peripheral masses may extend through an interlobar fissure or invade the chest wall to produce rib destruction.<sup>120</sup>

A frequently noted association in cases of blastomycosis presenting as either consolidation or a mass is the presence of 1–4 intermediate-sized nodules located in regions remote from the dominant focus (see Fig. 6-29).<sup>120</sup> A miliary pattern of pulmonary disease is not widely recognized as a manifestation of blastomycosis (Fig. 6-32).<sup>126</sup> *Awareness of this manifestation is important, because miliary blastomycosis is often fatal.*<sup>126</sup> It is estimated that this pattern is found in 4–28% of patients.<sup>127,128,146,150</sup> Miliary disease may develop abruptly, either after a normal chest radiograph or after a focal consolidation.<sup>165,168</sup> Pleural effusion commonly accompanies miliary disease. Thickening of pleura without an effusion is relatively frequent (2% and 48%) (see Fig. 6-29).<sup>123,127,128</sup> Although pleural effusions have been reported with a high frequency of 88%,<sup>129</sup> they are uncommon or rare. They are more frequent in severely ill patients.<sup>129</sup> When present, they tend to be small.

The most common presenting CT features, first reported in 16 patients,<sup>124</sup> were pulmonary masses, consolidation, or both. Masses were more commonly detected than consolidation (88% vs 56%), probably because of selection bias favoring patients with masses to undergo CT examinations (see Fig. 6-31). Masses were greater than 2 cm in diameter in 88% of cases and nodules 3 mm to 2 cm in diameter were present in 75% of cases. Air bronchograms were frequent in both consolidations (88%)





**Figure 6-31** Lung mass in blastomycosis. (A) PA chest radiograph of an asymptomatic 62-year-old man demonstrates an irregularly margined mass (arrows) above the hilum in the right upper lobe. (B) Chest CT image (lung window) reveals a lobulated opacity adjacent to the right main bronchus. Lobectomy with nodal dissection demonstrated granulomatous pneumonitis and lymphadenitis secondary to blastomycosis.

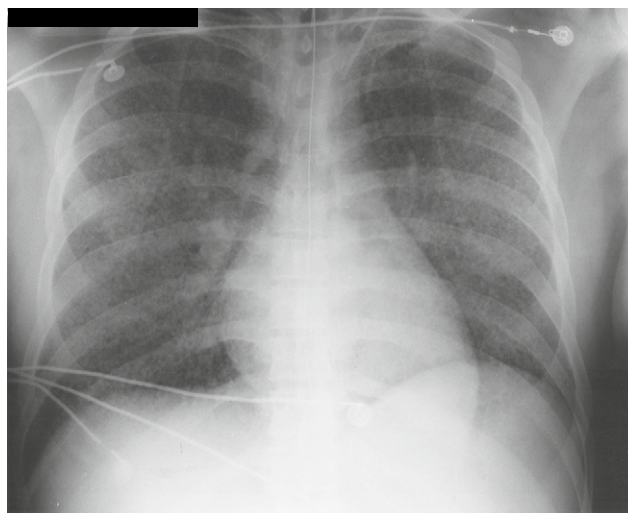
and masses (86%). These frequencies are much higher than those reported on radiographs.<sup>124</sup> Cavitation was seen in only 13% of patients and was limited to upper lobe disease (Fig. 6-33). In 75% of cases, parenchymal disease extended to the hilum. No lobar predilection was found for either mass lesions or consolidations. Miliary disease was found in one case (6%). Satellite lesions (intermediate-sized nodules in a lobe with consolidation or a mass) were commonly present (69%). Hilar or mediastinal lymph nodes were small (1–2 cm) and uncommon (25%). Pleural thickening was minimal and present in 25% of cases, whereas effusions were seen in 13%. No instances of chest wall invasion were found.

After treatment or spontaneous resolution, blastomycosis usually leaves signs of the previous infection. In Brown's series of 33 patients with long-term follow-up,<sup>120</sup> 15% of cases had fibrotic or fibronodular changes on chest x-ray. None had calcification in the lungs or lymph nodes. Hilar adenopathy and extrapulmonary involvement were rare in this series, and pleural effusion did not occur, although chest wall masses, rib destruction, and cutaneous fistulas are reported.<sup>124</sup>

The most common radiologic finding in children with pulmonary blastomycosis is also parenchymal airspace consolidation (89%), either in one lobe or multilobar, which rarely cavitates (11%). Pleural effusion (17%), hilar adenopathy (11%), and rib involvement (11%) are less common.<sup>130</sup>

## Candidiasis

Making the diagnosis of pulmonary candidiasis is difficult for several reasons. *Candida* species are part of normal oral and gastrointestinal flora,<sup>131</sup> and therefore sputum cannot be relied on for diagnosis. Because virtually all patients with *Candida* pneumonia are immunocompromised, the organism frequently colonizes the tracheobronchial tree and tissue invasion

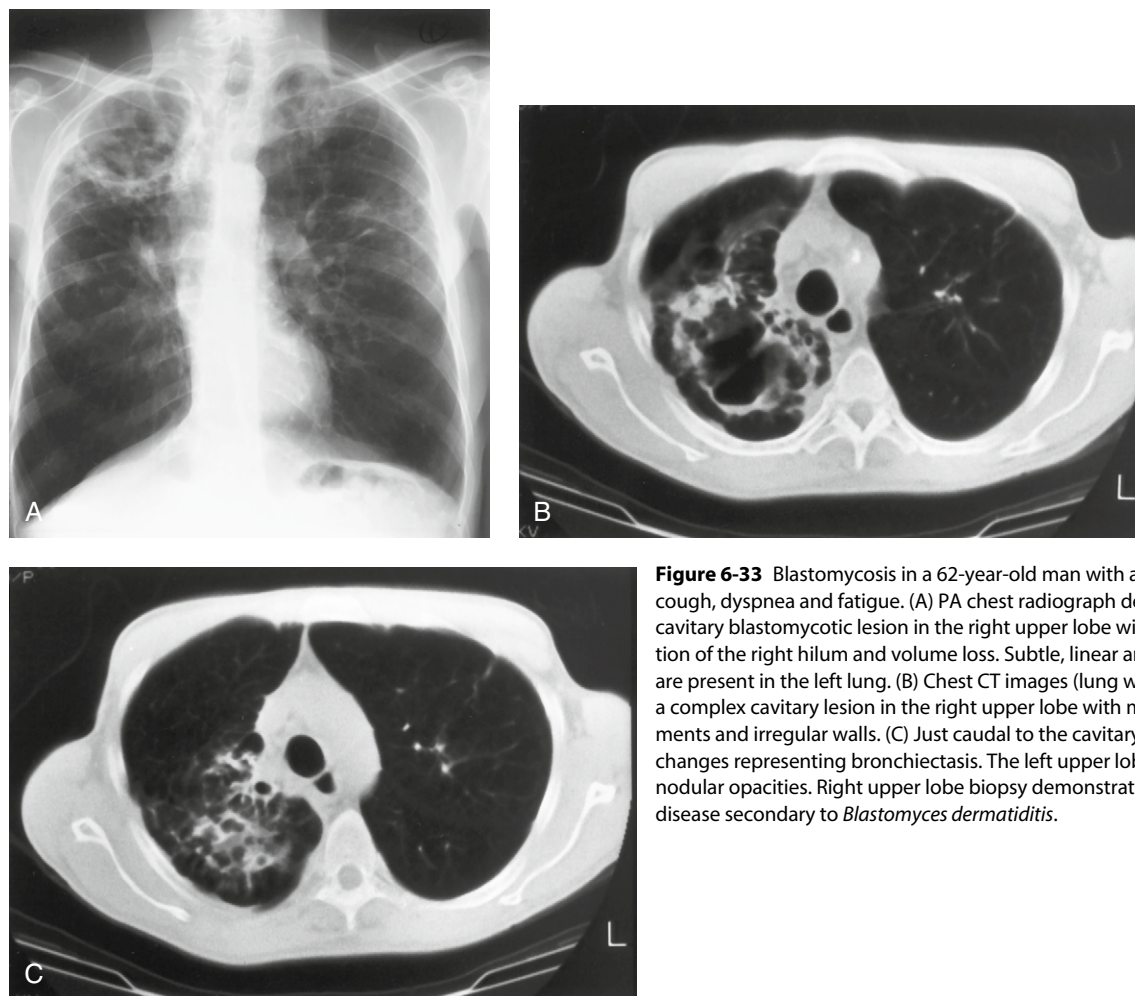


**Figure 6-32** Blastomycosis in a 30-year-old man with septic arthritis of the right knee who developed respiratory distress. AP bedside chest radiograph demonstrates bilateral, generalized miliary nodules without pleural effusion or lymphadenopathy. A feeding tube and left central line are in place. Open lung and skin biopsies demonstrated blastomycosis.

must be proven to exclude the possibility of contamination due to colonization. Accordingly, an invasive technique, bronchoscopic biopsy or surgery, is required to document tissue invasion. Because *Candida* species often co-exist in lung infected by bacteria, proving that the radiographic features of a pneumonia are due solely to *Candida* is extremely difficult.

In a retrospective study by Duke and Yale Universities over a 10-year period,<sup>132</sup> 20 severely immunocompromised patients with autopsy-proven *Candida albicans* as the sole organism





**Figure 6-33** Blastomycosis in a 62-year-old man with a 6-month history of cough, dyspnea and fatigue. (A) PA chest radiograph demonstrates a large cavitary blastomycotic lesion in the right upper lobe with cephalad retraction of the right hilum and volume loss. Subtle, linear and nodular opacities are present in the left lung. (B) Chest CT images (lung window) demonstrate a complex cavitary lesion in the right upper lobe with multiple compartments and irregular walls. (C) Just caudal to the cavitary lesion are “cystic” changes representing bronchiectasis. The left upper lobe contains linear and nodular opacities. Right upper lobe biopsy demonstrated granulomatous disease secondary to *Blastomyces dermatitidis*.

causing pneumonia were identified. *The most characteristic radiographic abnormality observed was airspace consolidation* (Fig. 6-34). Eleven cases (55%) also demonstrated a co-existent interstitial component (Fig. 6-35). Bilateral, non-segmental, homogeneous or patchy, poorly defined foci were present in 40% of cases (see Fig. 6-34). Lobar disease was bilateral in 40% and unilateral in 20%. Exudative pleural effusions were present in 25% of cases, but only three of 20 patients had infection limited to the lungs. None of the films demonstrated infiltrates with cavitation, adenopathy, or extension of pulmonary disease into the chest wall. In another study,<sup>133</sup> using autopsy records and chest radiographs of 14 infants with pulmonary candidiasis over a 12-year period, the typical radiographic appearance was also that of progressive airspace opacity. However, two cases with embolic pulmonary candidiasis exhibited cavitation on chest radiographs. Other investigators also described a diffuse miliary-nodular pattern on chest films,<sup>133</sup> which some investigators believe is an early manifestation of pulmonary candidiasis.<sup>173,174</sup> Interestingly, *Candida* and allergic bronchopulmonary candidiasis have been reported, but are rare.<sup>134</sup>

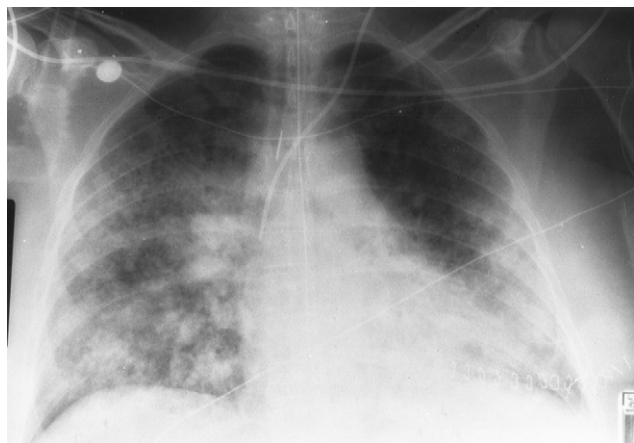
## Coccidioidomycosis

Pulmonary infection with coccidioidomycosis has been classified into four forms: primary, persistent primary, chronic progressive and disseminated. This organization best characterizes

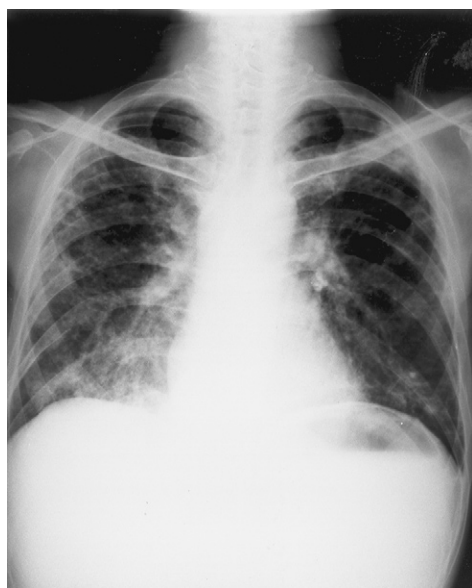
the variety of pathologic, radiologic, and clinical manifestations of thoracic coccidioidomycosis.

### Primary coccidioidomycosis

Like histoplasmosis, pulmonary coccidioidomycosis may be present in the absence of any radiographic abnormality, and most individuals with primary disease remain asymptomatic (60–80%). *Of patients who are symptomatic with primary disease, airspace consolidation is the most common radiographic finding* (Fig. 6-36).<sup>135</sup> The consolidation is typically segmental or subsegmental, and the degree of opacification ranges from dense and homogeneous to mottled and patchy.<sup>136</sup> In a series of patients hospitalized with pulmonary coccidioidomycosis, almost half (46%) had a segmental opacity, whereas 27% had a minimal (patchy, subsegmental) opacity.<sup>137</sup> Opacities of primary coccidioidomycosis are most commonly located within the lower lobes. Although pleuritic chest pain occurs in 70% with primary disease, in only 20% does a small pleural effusion develop.<sup>138</sup> Large effusions are uncommon (2–6%).<sup>138</sup> When present, an effusion usually accompanies a pulmonary opacity. *Pleural effusion in the absence of pulmonary disease is an uncommon presentation of coccidioidomycosis.* Most effusions resolve rapidly and completely.<sup>138</sup> Hilar adenopathy occurs in association with parenchymal coccidioidomycosis in 20% and rarely occurs without parenchymal disease. In a series of

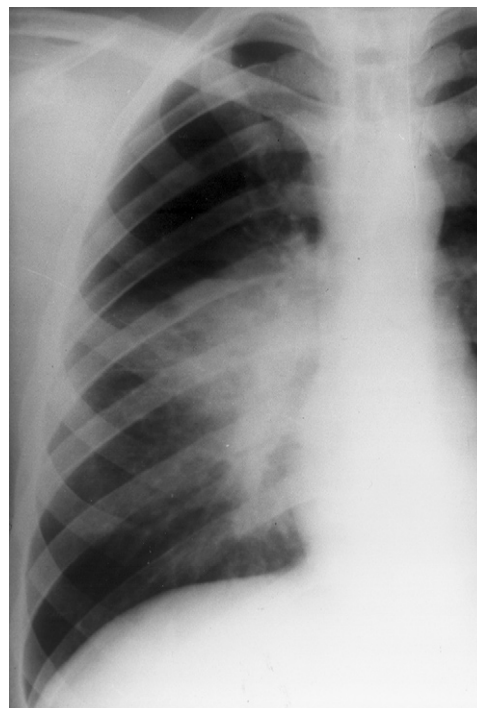


**Figure 6-34** *Candida* pneumonia in a leukemic with endotracheal tube, orogastric tube and central venous line. AP bedside chest radiograph shows generalized coalescing nodular airspace opacities. A recently performed open lung biopsy (skin staples overlie lower left chest) revealed *Candida albicans* pneumonia.



**Figure 6-35** Candidiasis in a 56-year-old man with chronic lymphocytic leukemia. PA chest radiograph shows bilateral reticulonodular interstitial pulmonary opacities. The right lung is more severely involved than the left.

59 patients hospitalized for coccidioidomycosis,<sup>138</sup> hilar adenopathy occurred in 19% and mediastinal adenopathy in 8.5%. Lymph node enlargement without concomitant pulmonary disease was present in only one case. Adenopathy is usually asymptomatic but can become bulky and compromise the tracheobronchial lumen, particularly in children.<sup>137</sup> *The small peripheral calcified pulmonary nodule, so common in histoplasmosis, is rarely present in coccidioidomycosis.*<sup>139</sup> In 85–95% of cases, the infiltrates and effusions in primary coccidioidomycosis resolve in 2–3 weeks.<sup>136,139</sup> However, adenopathy, especially mediastinal, may persist for months to years.<sup>139</sup>

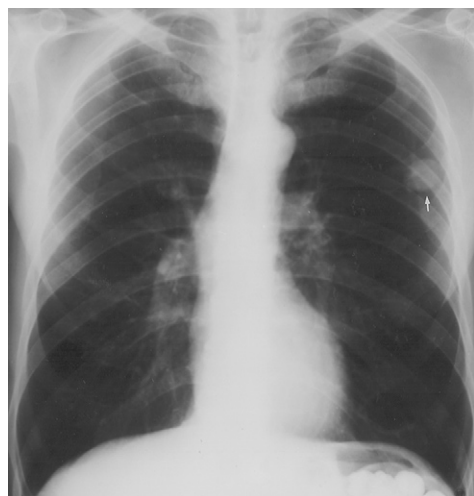


**Figure 6-36** Primary coccidioidomycosis. PA chest radiograph coned to the right lung of a 26-year-old man shows a segmental airspace consolidation overlying the hilum within the superior segment of the lower lobe.

#### Persistent primary coccidioidomycosis

In approximately 5% of patients with primary disease the pulmonary infection persists longer than 6 weeks and is termed persistent primary.<sup>135,136</sup> The most serious manifestation of persistent primary disease is progressive pneumonia during which infection spreads to large portions of the lung. This complication commonly affects immunosuppressed individuals and may be fatal. Foci of dense consolidation that develop as a consequence of persistent primary disease resolve very slowly, requiring many months.<sup>136</sup> A second manifestation of persistent primary disease is the development of pulmonary nodules (coccidioidoma) (Fig. 6-37). Coccidioidoma usually occur at the site of the resolving airspace consolidation as a single, sharply defined, spherical opacity 0.5–5 cm, typically in the mid or upper lungs.<sup>136</sup> Occasionally, multiple coccidioidoma develop.<sup>147,150</sup> A coccidioidoma is analogous to the histoplasma, but internal calcifications are uncommon.

*Cavitation is another hallmark of persistent primary disease.* It develops within the foci of consolidation (Fig. 6-38) and within coccidioidoma (Fig. 6-39). These cavities tend to occur in the upper lobes or the superior segments of the lower lobes. When the process of cavitation is incomplete, the wall is thick and irregular (see Fig. 6-39). *More commonly, cavitation is complete, resulting in a uniformly thin-walled cyst, known as a “grape skin cavity”* (see Fig. 6-38). These cavities may gradually change in size, presumably as a result of a check-valve phenomenon. Coccidioidoma may also remain stable for months, resolve completely, or break down and spread locally or disseminate. Rupture of the cavity into the pleural space occurs in 1.5–2.5% of patients with cavitory disease, and a coccidioidal empyema may accompany the spontaneous pneumothorax.<sup>140</sup>



**Figure 6-37** Coccidioidoma. PA chest radiograph of a 61-year-old asymptomatic man demonstrates a left upper lobe ovoid, non-calcified solitary pulmonary nodule (arrow). Biopsy demonstrated a fibrocasseous granuloma of coccidioidomycosis.

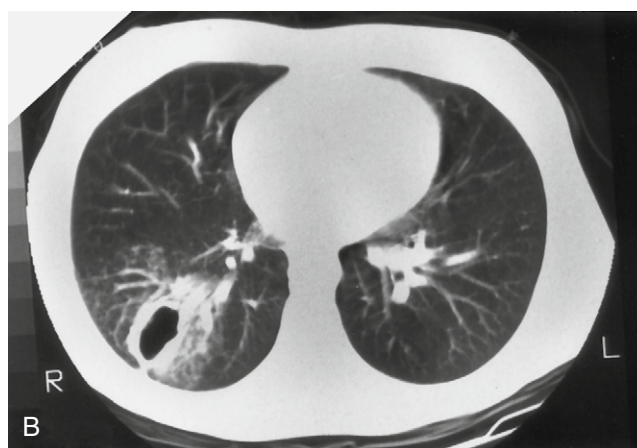
Rarely, fungus balls due to *Coccidioides immitis* or *Aspergillus* develop within coccidioidal cavities.<sup>139</sup>

#### Chronic progressive coccidioidomycosis

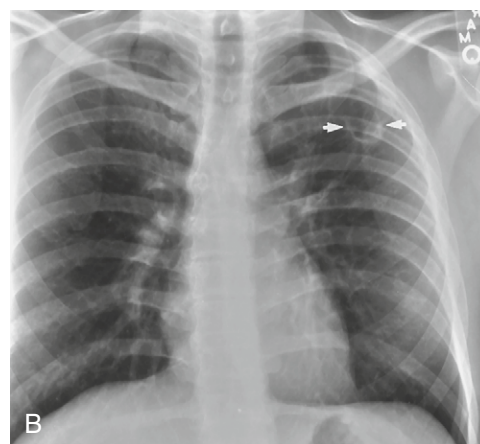
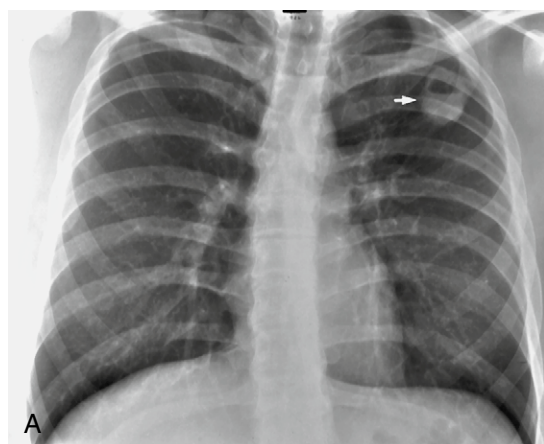
This advanced state of pulmonary coccidioidomycosis represents a relentless infection and occurs in less than 1% of cases with pulmonary disease.<sup>136</sup> Two types are recognized: chronic progressive fibronodular disease (Fig. 6-40) and chronic progressive necrotizing disease (Figs 6-41 and 6-42). Chronic progressive disease may occur as a direct continuation of primary pulmonary disease or as a temporally remote reactivation of apparently stable disease. This form of disease typically occurs in the upper lobes and mimics cavitory tuberculosis (see Fig. 6-40).

#### Disseminated coccidioidomycosis

Spread to extrathoracic locations occurs in approximately 1/6000 patients hospitalized with coccidioidomycosis.<sup>136</sup> Dissemination usually occurs early in the course of persistent primary disease<sup>136</sup> and may be fatal. Filipinos, African-Americans, Mexican-Americans, Native Americans, and



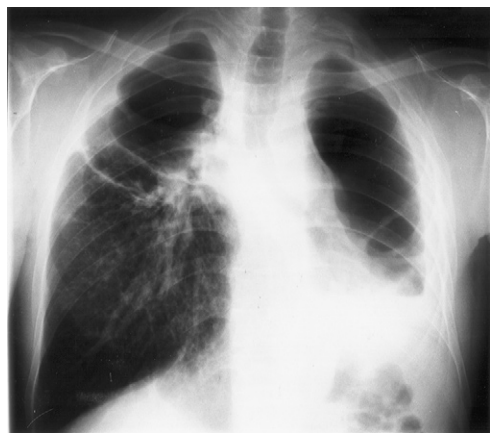
**Figure 6-38** Persistent primary coccidioidomycosis in a 29-year-old woman with hepatic failure and chronic right lower lobe disease. (A) PA chest radiograph demonstrates a large thin-walled right lower lobe cavity (arrows) with consolidation of the adjacent lung. (B) Chest CT image (lung window) reveals a 3.5 cm right lower lobe cavity with surrounding airspace disease. The wall of the cavity is relatively thin but irregular. Right lower lobectomy demonstrated necrotizing granulomatous disease and spherules of *Coccidioides immitis*.



**Figure 6-39** Persistent primary coccidioidomycosis in a 34 year old with chronic coccidioidomycosis. (A) PA chest radiograph demonstrates a cavitary mass (arrow) of the left upper lobe with an air-fluid level. (B) PA chest radiograph obtained 9 months later shows a residual thin-walled cavity (arrow) in the left upper lobe and resolution of the air-fluid level.



immunocompromised patients are at risk for dissemination.<sup>136</sup> In the presence of pulmonary coccidioidomycosis, enlargement of mediastinal lymph nodes often heralds dissemination (see Fig. 6-42).<sup>136</sup> A miliary pattern indicates hematogenous dissemination (Fig. 6-43).<sup>136</sup>



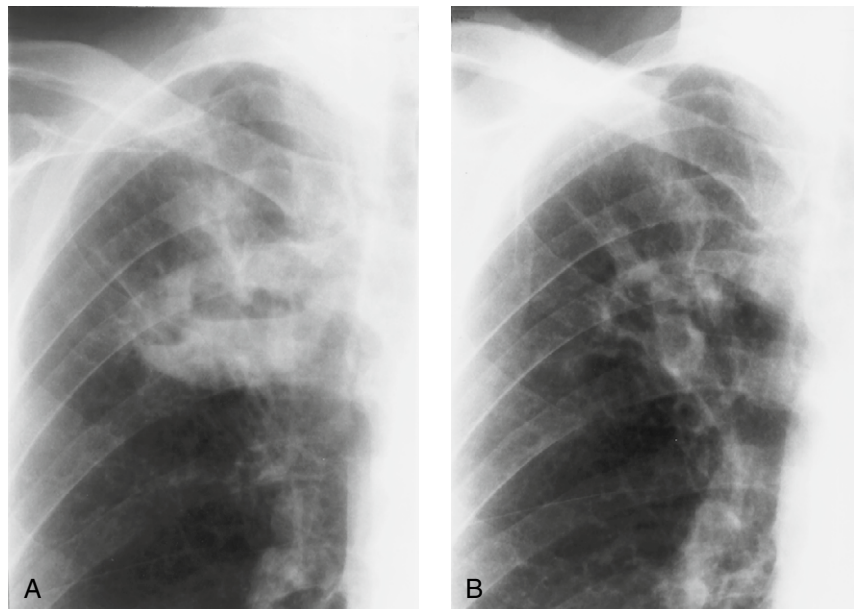
**Figure 6-40** Chronic progressive coccidioidomycosis in a 34-year-old HIV-negative man with no risk factors for AIDS who had been coughing up *Coccidioides immitis* spherules and hyphal forms. His PPD skin test was negative while controls were positive, and sputum did not contain AFB organisms. PA chest radiograph shows a large thin-walled cavity in the right upper lobe with associated volume loss and thickening of surrounding pleura. A huge cavity in the left upper thorax which contains an air-fluid level and is surrounded by pleural thickening represents an empyema with bronchopleural fistula and hydropneumothorax left. The upper lobe is compressed against the mediastinum, and the left lower lobe is partially collapsed and contains bronchiectasis.

## Cryptococcosis

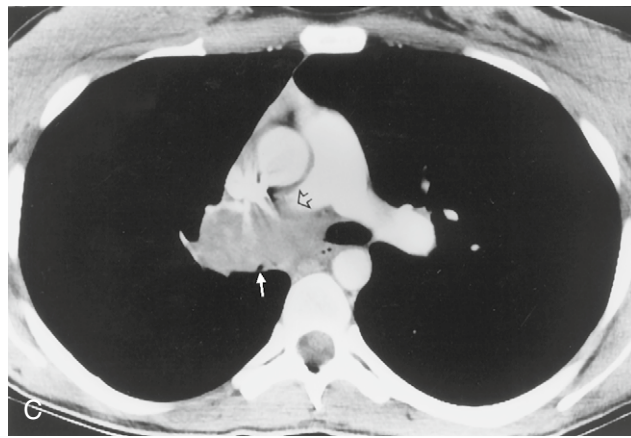
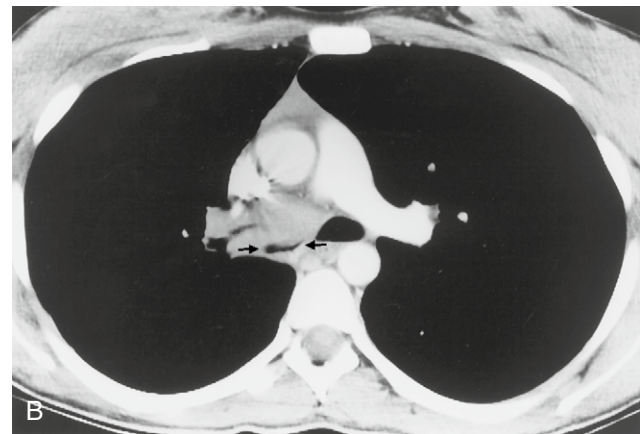
Pulmonary involvement is estimated to occur in 10–39% of patients with cryptococcosis.<sup>141</sup> Although the radiologic features of pulmonary cryptococcosis are varied, most authors suggest that pulmonary disease creates three patterns.<sup>142</sup> They are mass, airspace consolidation, and multiple, bilateral opacities, similar to the patterns caused by blastomycosis. *Pulmonary cryptococcosis manifests as a solitary mass or multiple pulmonary nodules in 42–89% of patients (Fig. 6-44).*<sup>183,185</sup> A single mass is much more common, typically located in the lung periphery and variable in diameter.<sup>142</sup> The margins of the mass may be either well circumscribed (see Fig. 6-44) or ill defined (Fig. 6-45). One author<sup>143</sup> reported that the most characteristic feature was a pulmonary opacity with both features. On one chest film projection, disease appeared as a consolidation, whereas on the orthogonal projection, it appeared as a mass. Multiple masses and cavitation are uncommon (see Fig. 6-45).<sup>142,143</sup>

Cryptococcosis produces a pattern of airspace consolidation with approximately equal frequency to that of a solitary mass (Fig. 6-46).<sup>144</sup> *Air bronchograms are uncommon.*<sup>142</sup> Cavitation within the consolidation and lymphadenopathy is present in approximately 8% of cases.<sup>144</sup> Pleural effusion is uncommon in any pattern of cryptococcosis. A third pattern that is commonly produced is that of bilateral, multiple small nodules or masses or diffuse reticulonodular opacities and associated with systemic dissemination.

Greater than 50% of symptomatic patients are immunocompromised. In a series of 24 patients reviewed by Khoury et al,<sup>141</sup> the radiographic abnormalities in immunocompromised patients differed from those occurring in immunocompetent patients. Although single and multiple nodules were the most frequently observed abnormality in both groups, this



**Figure 6-41** Chronic progressive coccidioidomycosis in a 50-year-old man with chronic coccidioidomycosis. (A) PA chest radiograph coned to the right upper lobe shows a complex cavitary focus of airspace opacity with multiple air-fluid levels. (B) Ten months later, a PA chest radiograph of the same area shows significant reduction in size of the cavitary disease and interval development of fibronodular opacities.

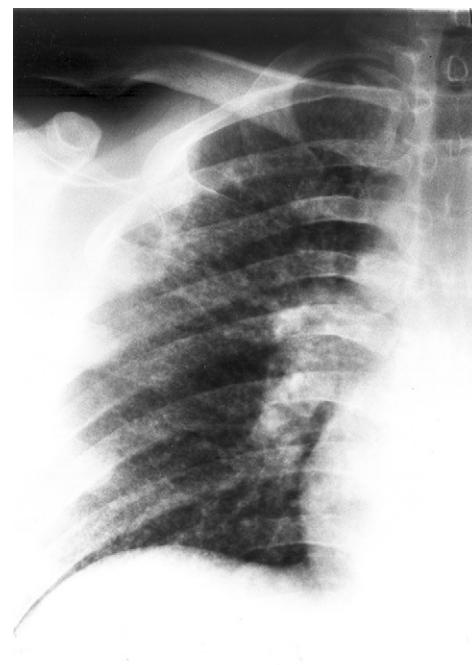


**Figure 6-42** Chronic progressive coccidioidomycosis in a 17-year-old girl with fever, productive cough, and weight loss over several months. Coccidioidomycosis had been diagnosed 3 years earlier. (A) PA chest radiograph shows right lower lobe collapse, a small right pleural effusion, and enlarged right paratracheal adenopathy. (B,C) Chest CT images (soft tissue window) reveal infiltrating soft tissue mass in the subcarinal, pre-carinal and right hilar areas causing a slit-like narrowing of the right main bronchus (black arrow), severe stenosis of the bronchus intermedius (white arrow), and obstruction of the right pulmonary artery (open arrow). Pneumonectomy confirmed an infiltrating, fibrotic soft tissue mass. *Coccidioides immitis* was isolated from the surgical specimen.

abnormality was more frequent in immunocompetent patients. Forty percent of immunocompromised patients demonstrated more extensive consolidations. *Cavitation, adenopathy, and pleural effusion were seen only in the compromised patients.* In recent years, *cryptococcosis has been the most common opportunistic fungal infection in AIDS patients*, representing between 2% and 15% of all pneumonias in this population. In a series<sup>145</sup> of seven patients with pulmonary cryptococcosis and AIDS, none showed an alveolar consolidation or a large nodule or mass on radiographs. Instead, four of seven demonstrated interstitial opacities, two with associated adenopathy, and one exhibited a focal nodular infiltrate. Two additional studies revealed hilar or mediastinal adenopathy alone. A unilateral pleural effusion was the only abnormality in the seventh patient. Three of the seven patients with disseminated cryptococcosis died.

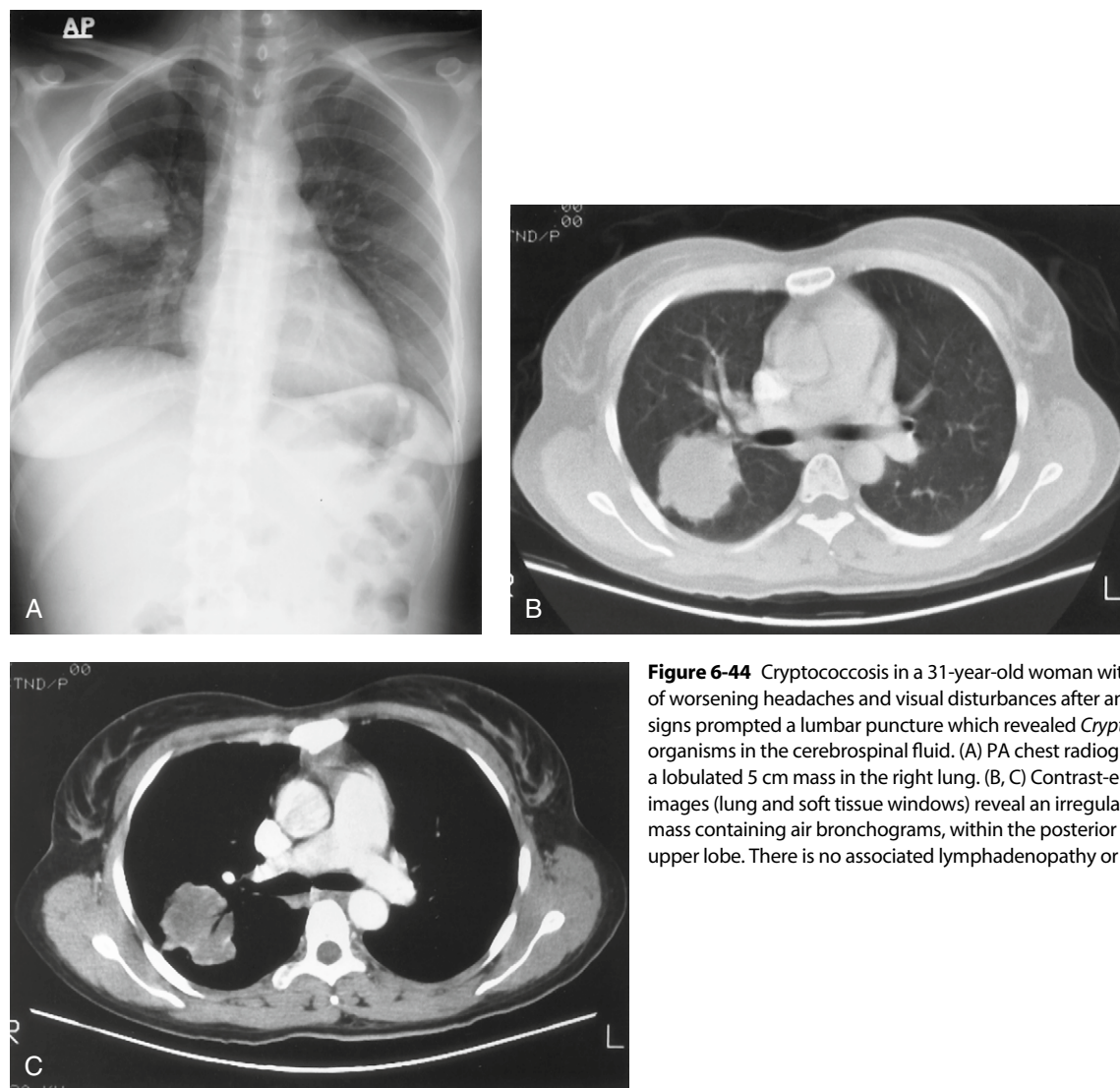
## Histoplasmosis

The radiologic features of pulmonary histoplasmosis are varied and fortunately, 90–95% of individuals exposed to *Histoplasma* organisms are asymptomatic and have normal radiographs. Only 10–25% of patients with subclinical episodes of histoplasmosis demonstrate active pulmonary disease on chest radiographs. Thoracic histoplasmosis manifests in five forms: primary pneumonia, reinfection, chronic pulmonary infection, disseminated and chronic mediastinal disease.

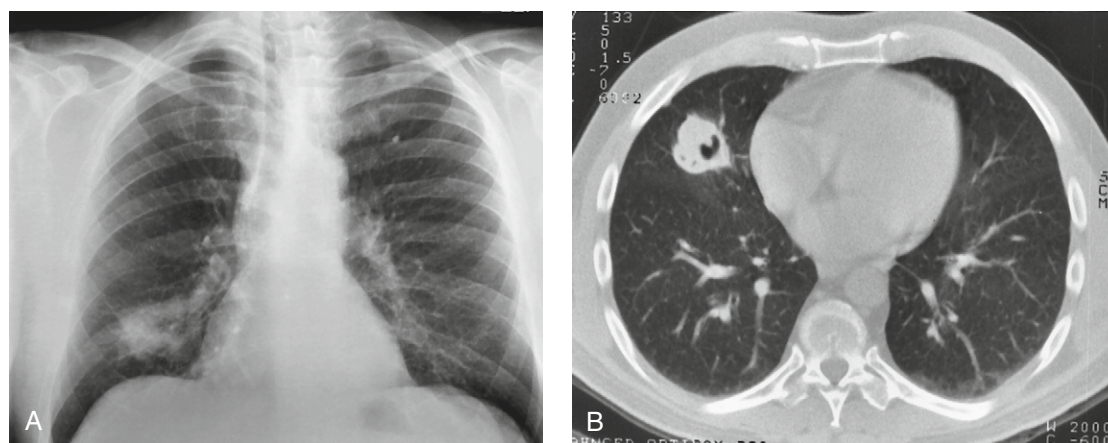


**Figure 6-43** Miliary coccidioidomycosis. PA chest radiograph coned to the right upper lobe demonstrates a generalized micronodular pattern associated with lymphadenopathy in the right hilum and right paratracheal mediastinum.



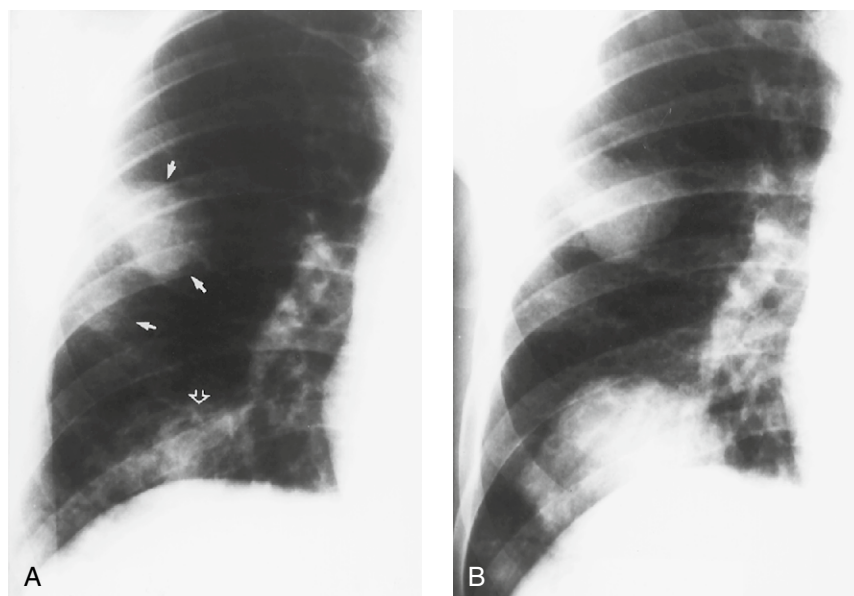


**Figure 6-44** Cryptococcosis in a 31-year-old woman with a 1-month history of worsening headaches and visual disturbances after an assault. Meningeal signs prompted a lumbar puncture which revealed *Cryptococcus neoformans* organisms in the cerebrospinal fluid. (A) PA chest radiograph demonstrates a lobulated 5 cm mass in the right lung. (B, C) Contrast-enhanced chest CT images (lung and soft tissue windows) reveal an irregular, low-attenuation mass containing air bronchograms, within the posterior segment of the right upper lobe. There is no associated lymphadenopathy or pleural effusion.

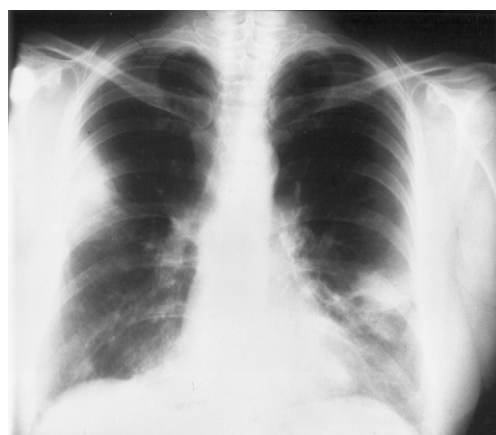


**Figure 6-45** Cryptococcosis in an asymptomatic 62-year-old man. (A) PA chest radiograph shows a large, irregular right middle lobe mass. (B) Contrast-enhanced chest CT image (lung window) reveals a cavitary mass with thick irregular walls and lobulated borders. Bronchoscopy and needle biopsy demonstrated *Cryptococcus neoformans* organisms. On oral fluconazole treatment almost complete resolution of the mass occurred.





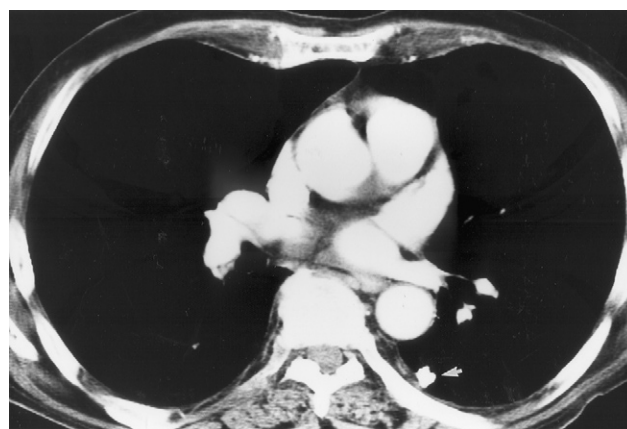
**Figure 6-46** Cryptococcosis. (A) PA chest radiograph coned to the right lower lung demonstrates a mass with adjacent airspace opacity (*arrows*) in the lateral aspect of the lung as well as a subtle, rounded focus airspace disease (*open arrow*) just above the diaphragm. (B) Coned PA chest radiograph taken 18 months later reveals resolution of the airspace disease adjacent to the original mass as well as the evolution of a second mass from the focus of airspace disease above the diaphragm.



**Figure 6-47** Primary histoplasmosis pneumonia. PA chest radiograph of 58-year-old woman shows bilateral, peripheral subsegmental lung opacities. No effusion or lymphadenopathy is identified.

### Primary pneumonia

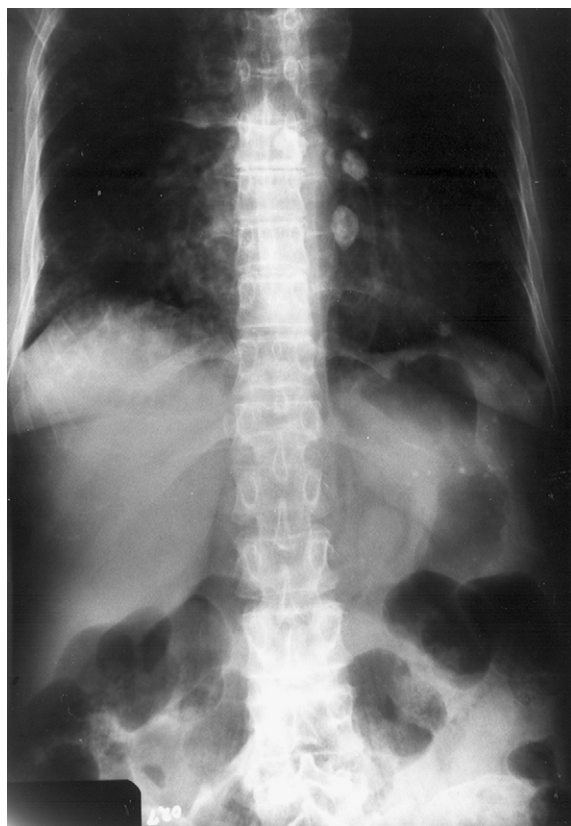
Within 3 weeks of previously unexposed hosts inhaling infectious microconidia, pulmonary opacities may appear.<sup>146</sup> Approximately 60–70% of chest radiographs that show signs of infection demonstrate one or more ill-defined non-segmental foci of homogeneous consolidation (Fig. 6-47),<sup>176,177</sup> which rarely demonstrate cavitation.<sup>147</sup> Opacities of primary pneumonia more commonly affect the lower lobes than upper lobes, and they may clear in some areas and progress in others.<sup>148</sup> An accompanying parapneumonic effusion occurs in only 5% of cases.<sup>149</sup> Eventually the opacity clears and the lung appears normal or develops a subcentimeter peripheral, well-circumscribed nodule in the subpleural region. The nodule may contain central calcification or be totally calcified (Ghon lesion) (Fig. 6-48) and is typically associated with calcification of the draining hilar lymph nodes (Rhanke complex) (Fig. 6-49). Although this radiographic pattern is similar to the Ghon complex of pulmonary tuberculosis, the calcified pulmonary nodules and hilar lymph nodes in histoplasmosis tend



**Figure 6-48** Calcified histoplasmosis Ghon lesion. Contrast-enhanced chest CT image (soft tissue window) reveals a sharply defined, irregular calcified opacity adjacent to the pleura of the left lower lobe representing a calcified granuloma (*arrow*). (The white areas within mediastinal and hilar structures represent normal enhanced vasculature.)

to be larger. Pulmonary calcifications larger than 4 mm and calcified lymph nodes larger than 10 mm are much more typical of histoplasmosis than of tuberculosis (see Fig. 6-49).<sup>150</sup>

Instead of resolving or developing a small peripheral nodule, focal parenchymal disease may heal by forming a mass, a histoplasmoma. A teaching center in an area endemic for histoplasmosis reported only 17 histoplasmomas resected to exclude lung carcinoma over 15 years.<sup>151</sup> On chest radiographs, a histoplasmoma usually manifests as a peripheral, solitary, spherical, smooth opacity and measures 5 mm to 3 cm in diameter (Fig. 6-50). Multiple histoplasmomas are a well-recognized occurrence.<sup>151</sup> The number of masses seldom exceeds four or five, and they often exhibit considerable variation in size. Most histoplasmomas develop in the lower lobes. Although not always visible on plain radiographs, most contain calcification, typically located centrally (see Fig. 6-50), producing the “target” sign (Fig. 6-51), believed to be virtually pathognomonic of a histoplasmoma. Occasionally, a lamellar

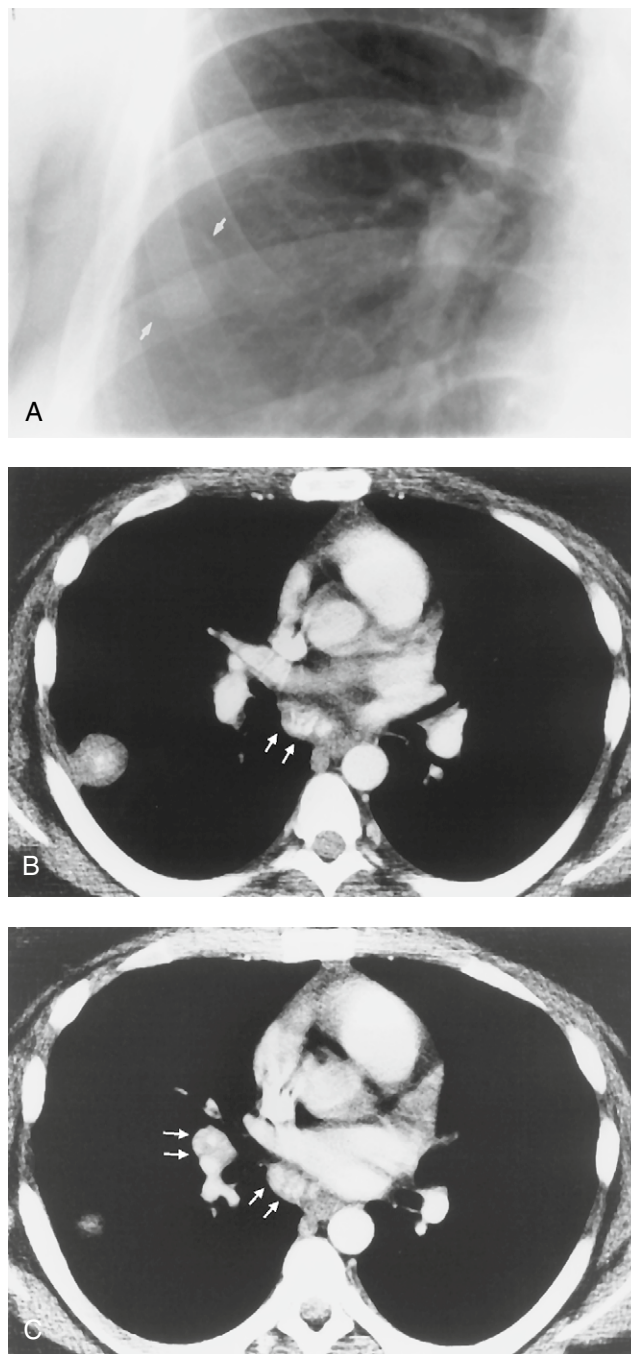


**Figure 6-49** Calcified histoplasmosis Rhanke complex. AP chest radiograph demonstrates a subtle calcified nodule in the left lower lobe just above the dome of the diaphragm and several large calcified left hilar and subcarinal lymph nodes. Tiny calcifications within the left upper quadrant of the abdomen represent splenic granuloma from healed systemic dissemination.

pattern of concentric calcific rings accompanies the central calcification (Fig. 6-52). Enlargement of a histoplasma may prompt surgical excision to exclude a lung carcinoma.

Individuals who inhale large quantities of organisms from sites containing heavy infestation of *Histoplasma* develop a pattern similar to the micronodular appearance of reinfection disease. Initially chest radiographs are normal for a week or so. Thereafter, bilaterally symmetric, generalized small, discrete, nodular lung opacities appear (Fig. 6-53). Lesions are slightly larger than those of typical cases of reinfection histoplasmosis, measuring from 3–4 mm to more than 1 cm in diameter.<sup>148</sup> In cases of massive inhalation of organisms, hilar lymphadenopathy is typically present (see Fig. 6-53). Within 2–8 months these resolve or fibrose, with many becoming diffusely calcified. Increasing the number of organisms in the inhalational exposure lengthens the time to resolution of the adenopathy. The radiograph may display numerous small calcified pulmonary nodules (Fig. 6-54).<sup>148</sup>

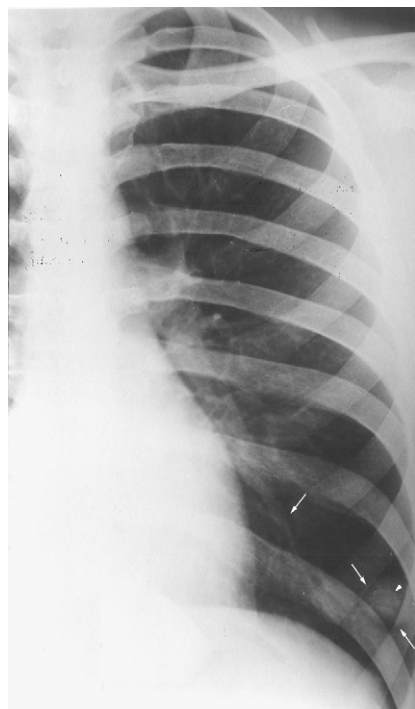
*Pulmonary disease is accompanied by hilar lymph node enlargement in approximately 60% of cases.*<sup>149</sup> Less commonly (10–27% of patients),<sup>149</sup> enlarged nodes without parenchymal disease may be identified in one or both hilar regions (Fig. 6-55) and occurs more frequently in children than in adults.<sup>152</sup> Although adenopathy usually produces no clinically important consequences, it may cause compression of airways and lobar



**Figure 6-50** Histoplasma. (A) PA chest radiograph coned to the right mid lung demonstrates a sharply margined spherical opacity (arrows) adjacent to the pleura. No internal calcifications or cavitations are identified. (B,C) Thin-section, contrast-enhanced chest CT images reveal calcifications within the mass (arrows), creating a “target” appearance surrounded by curvilinear lamellar calcifications. Note enlarged hilar right hilar and subcarinal lymph nodes which also contain calcifications (arrows).

collapse.<sup>146</sup> This most commonly affects the middle or a lower lobe.<sup>146</sup> With time, adenopathy typically regresses completely. During healing, affected lymph nodes commonly develop calcification and are usually an incidental finding on a chest radiograph. Rarely, a calcific focus erodes into the bronchus,

and is termed a broncholith and may be associated with hemoptysis, collapse, postobstructive pneumonia, and, rarely, lithoptysis. CT strongly suggests the diagnosis of broncholithiasis when the following findings are present: calcified lymph node within or adjacent to a bronchus; signs of bronchial obstruction-atelectasis, mucoid impaction, bronchiectasis, air trapping, or airspace disease; and absence of an associated hilar mass (Fig. 6-56).<sup>150</sup>



**Figure 6-51** Histoplasmosis. PA chest radiograph coned to the left lung of an asymptomatic 20-year-old man demonstrates a 2 cm ovoid nodule (arrows) with dense central calcification (arrowhead) just above the costophrenic angle. Wedge resection revealed a histoplasmosis.

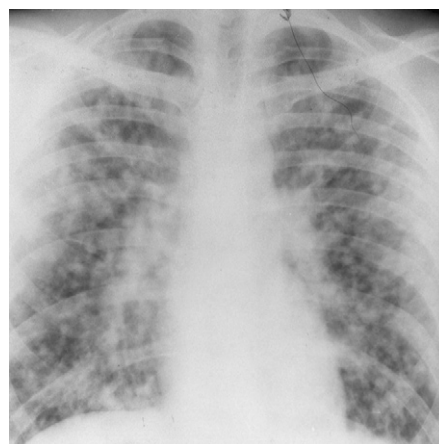
### Reinfection

The radiographic appearance is different from that of primary disease. Typically, reinfection manifests as a bilaterally symmetric micronodular process. Individual 1–2 mm nodules may coalesce (Fig. 6-57). Adenopathy and pleural effusions are usually absent.<sup>153</sup>

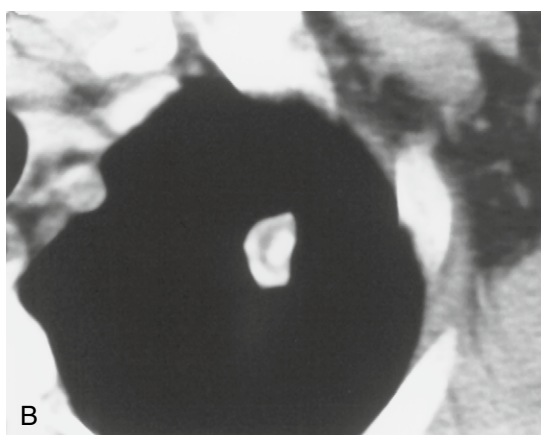
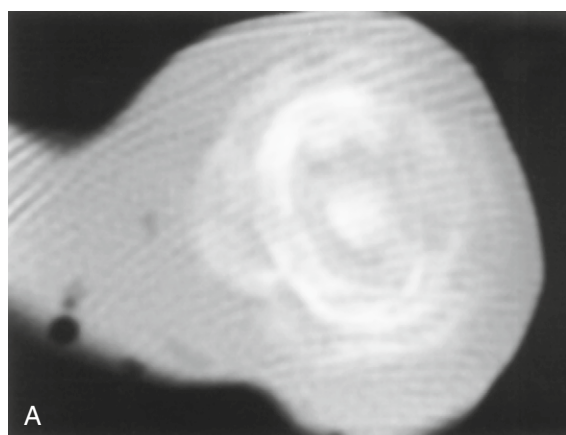
### Disseminated disease

Infection may extend beyond the thorax. Most individuals so affected have a benign, self-limited infection. However, some, particularly children <1 year or patients older than 50 years and the immunosuppressed, are prone to systemic dissemination. In these instances the lungs may exhibit a miliary pattern (1–2 mm) of generalized nodules or reticulonodular interstitial opacities.

Disseminated histoplasmosis represents one of the more frequently observed infections in AIDS patients who live in endemic regions. Conces et al reported the radiographic



**Figure 6-53** Massive inhalation of *Histoplasma* organisms. PA chest radiograph of a 32-year-old man who became symptomatic after cleaning chicken coops demonstrates a bilateral, generalized ill-defined nodular pneumonia with probable hilar lymphadenopathy.

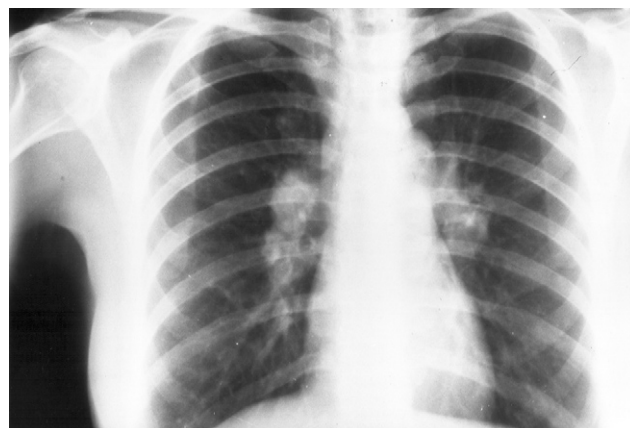


**Figure 6-52** Histoplasmosis. (A) Chest CT image (soft tissue window) of a resected portion of lung demonstrates a sharply defined, rounded mass with dense central calcifications, surrounded by rings of lamellar calcifications. (B) Chest CT image (soft tissue window) through the left upper lobe of another patient reveals a sharply defined ovoid mass with dense "target" central calcification, surrounded by a single lamellar calcification.

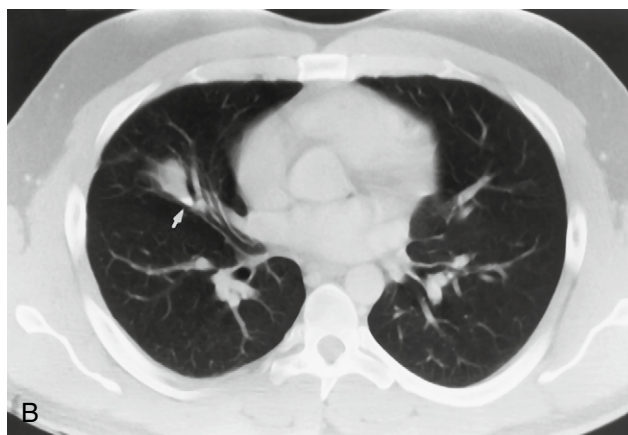
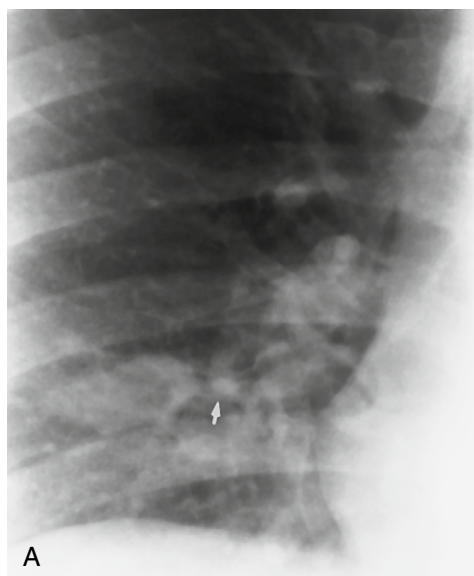




**Figure 6-54** Healed multifocal pulmonary granuloma. PA chest radiograph of a 41-year-old woman with a history of histoplasmosis demonstrates innumerable small punctate pulmonary calcifications. Calcified mediastinal lymph nodes are also present.



**Figure 6-55** Histoplasmosis with lymphadenopathy. PA chest radiograph demonstrates enlarged lymph nodes in both hilar and the right paratracheal and aorticopulmonary regions of the mediastinum. Lymphadenopathy eventually resolved.



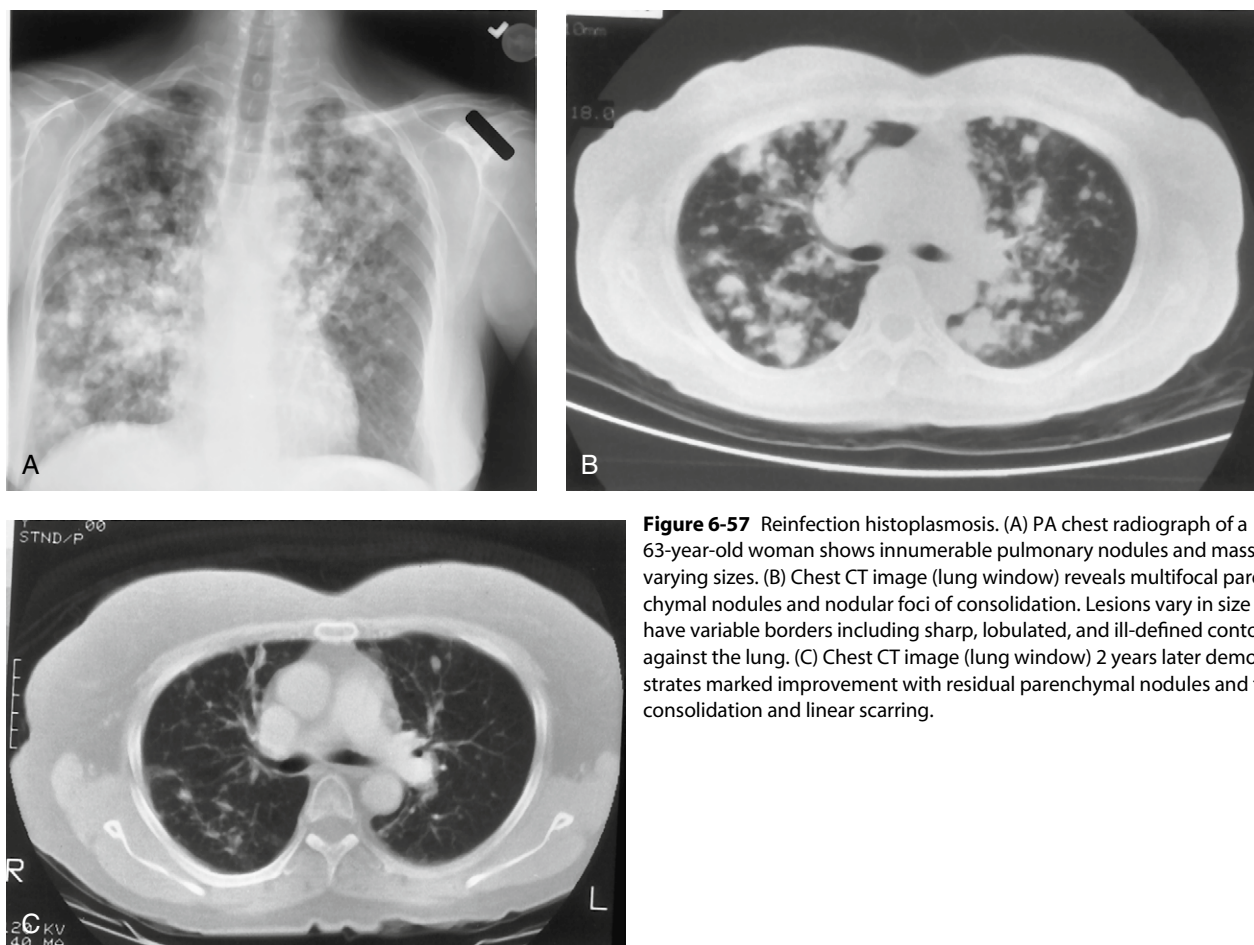
**Figure 6-56** Histoplasmosis with broncholith. (A) Coned PA chest radiograph demonstrates a subtle ovoid opacity just above the diaphragm. A small calcification (arrow) lies just medial to the opacity. (B) Chest CT image (lung window) through opacity demonstrates a small calcification at the posteromedial aspect of the opacity. Calcification represents a broncholith (arrow) within a subsegmental bronchus of the right middle lobe.

findings in 50 AIDS patients with disseminated histoplasmosis.<sup>154</sup> Almost half (46%) had no evidence of lung involvement on chest radiographs. Of those with radiographically visible pulmonary disease, multiple nodules were the most common finding. In all but one case, nodules were 3 mm or smaller in size, and involved all lung zones. Small pleural effusions were present in 10% of all AIDS patients with disseminated histoplasmosis, and adenopathy was seen in 6%.

#### Chronic pulmonary histoplasmosis

Occasionally, histoplasmosis develops a chronic form in patients with lungs previously damaged by emphysema and most frequently occurs within the upper lobes.<sup>153</sup> The inflammatory process is believed to be due to a hypersensitivity reaction to the organism rather than to an infection and is usually self-limited.<sup>150</sup> The inflammation may create areas of

opacification or reticulonodular infiltrates, particularly in the apical and posterior segments. This can lead to lung necrosis, vascular occlusion, and cavity formation.<sup>148</sup> Linear stranding extends from areas of inflammation to the hila. Extension into adjacent segments with progressive scarring, often with cavity formation, and volume restriction can develop. Bronchiectasis may also occur. The radiographic appearance mimics postprimary tuberculosis, but several important differences exist (Fig. 6-58). Chronic pulmonary histoplasmosis predominantly occurs in middle-aged men with emphysema and rarely affects women. True cavity formation is rare, and the inflammatory process is usually self-limited.<sup>150</sup> The destroyed lung becomes susceptible to recurrent bacterial pneumonias and abscess formation, and the patient may have hemoptysis, severe pulmonary insufficiency, and cor pulmonale.<sup>146</sup>



**Figure 6-57** Reinfection histoplasmosis. (A) PA chest radiograph of a 63-year-old woman shows innumerable pulmonary nodules and masses of varying sizes. (B) Chest CT image (lung window) reveals multifocal parenchymal nodules and nodular foci of consolidation. Lesions vary in size and have variable borders including sharp, lobulated, and ill-defined contours against the lung. (C) Chest CT image (lung window) 2 years later demonstrates marked improvement with residual parenchymal nodules and foci of consolidation and linear scarring.

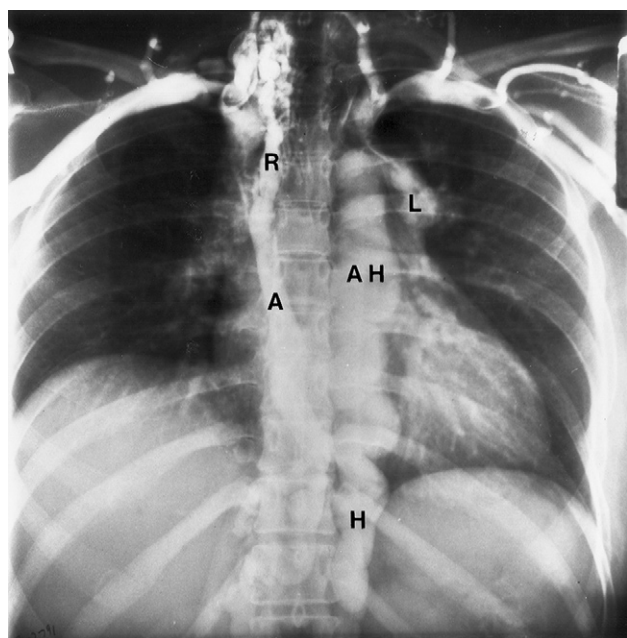
### Chronic mediastinal histoplasmosis

Rarely with granulomatous inflammation, the mediastinal lymph nodes soften and coalesce into a large matted encapsulated mass, known as a mediastinal granuloma.<sup>153</sup> These may evolve into masses 8–10 cm in diameter. Occasionally these masses contain central calcification.<sup>152</sup> CT scans of mediastinal granuloma reveal one or more large, heterogeneous masses of soft tissue attenuation, usually in the paratracheal or subcarinal areas. Central low attenuation due to necrosis with enhancing septae and punctate calcifications may be present.<sup>155</sup> Although the mass is not locally invasive, it may encroach on adjacent structures, esophagus and trachea. With time, the adenopathy can be expected to regress. However, the inflammatory process may extend into the pericardium and may cause granulomatous pericarditis, particularly in young adults.<sup>146</sup> The pericardial effusion may enlarge the cardiac silhouette. As chronic inflammation progresses, curvilinear calcifications may develop within the pericardium, resulting in constrictive pericarditis. Additional findings of constrictive pericarditis by CT or MR include enlargement of the right ventricle, right atrium, and vena cava, flattening or leftward convexity of the interventricular septum (paradoxical septal motion), and a normal or small left atrium and left ventricle.

Fibrosing mediastinitis (sclerosing mediastinitis) represents a less frequent but much more severe complication of



**Figure 6-58** Chronic pulmonary histoplasmosis. PA chest radiograph of a 34-year-old man shows complex cavitary disease within the apical areas of the upper lobes. Airspace consolidation extends into other segments of the right upper lobe. Subtle tubular lucencies within the consolidation suggest bronchiectasis. Note the markedly enlarged appearing lungs consistent with generalized emphysema. A left upper lobectomy after therapy revealed active histoplasmosis with caseating granuloma.



**Figure 6-59** Fibrosing mediastinitis. Bedside AP chest radiograph accomplished during a bilateral upper extremity venogram demonstrates obstruction of the superior vena cava and both brachiocephalic veins. Note the enlarged collateral vessels at the base of the right neck filling the right superior intercostal vein (R), and the markedly enlarged left superior intercostal vein (L), azygous (A), hemiazygous (H), and accessory hemiazygous (AH) veins.

histoplasmosis than mediastinal granuloma.<sup>156</sup> Although the two sequelae of mediastinal histoplasmosis are similar, it is generally accepted that mediastinal granuloma does not evolve into fibrosing mediastinitis.<sup>148</sup> An exuberant fibrogenesis extends beyond the capsule of affected lymph nodes and invades and constricts adjacent structures, particularly the superior vena cava (SVC), esophagus, airways, and pulmonary vessels. Obstruction of the SVC is the most common vascular complication (Fig. 6-59). Rarely the pulmonary arteries or veins become affected.<sup>157</sup> In a series of 33 patients evaluated by multiple radiologic modalities, bronchial narrowing was present in 33%, pulmonary obstruction was noted in 18%, esophageal obstruction in 9%, and superior vena cava obstruction in 39%.<sup>158</sup> Radiographically, sclerosing mediastinitis may demonstrate no abnormality.

The most common radiographic abnormality is a localized, sharply defined mass that causes a focal convex curvature of the mediastinal pleura into the adjacent lung (Fig. 6-60). The mass is usually located along the right paratracheal portion of the mediastinum. CT and MR reveal a poorly margined soft tissue mass (see Fig. 6-60), typically near the tracheal carina or the trachea. MRI helps differentiate the infiltrating fibrotic mass of fibrosing mediastinitis from a malignancy. On T2WI images the internal fibrotic tissue is lower in signal intensity than paraspinal musculature and is generally much lower than the signal created by a malignancy.<sup>159</sup> Radiographs may reveal calcified hilar and mediastinal lymph nodes, but CT is more sensitive than radiographs in identifying the mediastinal mass and central calcifications. When the mass narrows a pulmonary artery, diminished blood flow results in reduced lung

volume and mediastinal shift to the affected side (Fig. 6-61). The presence of extensive calcifications within enlarged matted lymph nodes is specific for fibrosing mediastinitis and helps to exclude the possibility of an infiltrating malignancy.<sup>158</sup> CT and MR examinations demonstrate vascular encasement from soft tissue and reduced or absent blood flow (see Figs 6-60, 6-61).<sup>158,160</sup> Radionuclide ventilation-perfusion studies show unilateral absence of perfusion with mildly abnormal ventilation (see Fig. 6-60). Pulmonary venous obstruction may create an appearance of unilateral venous hypertension.

## Paracoccidioidomycosis

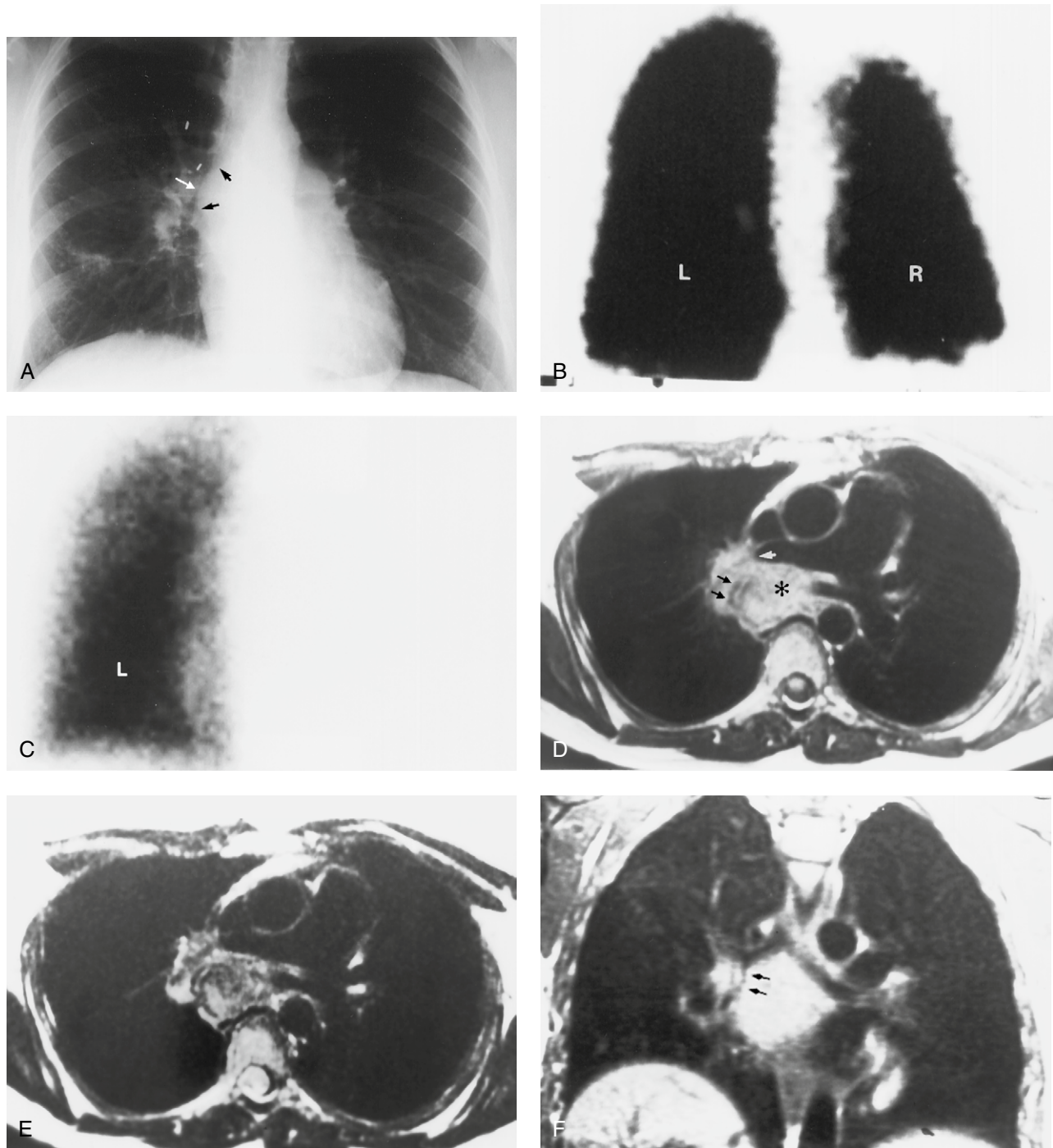
Paracoccidioidomycosis is the most common systemic mycosis in Latin America.<sup>161</sup> Only 11 cases were reported by 1977 in the United States.<sup>162</sup> In endemic areas, exposure is acquired through inhalation<sup>161</sup> and occurs at an early age. Although skin test positivity approaches 50% by the end of the second decade of life in some endemic regions of Brazil,<sup>163</sup> symptoms are often absent or minimal, non-specific and short-lived. Initial exposure to the organism does not lead to immediate pulmonary consequences. Prevalence of progressive forms of paracoccidioidomycosis in children is low (4–9% of all cases).<sup>163</sup> Children typically present with lymphadenitis, gastrointestinal or cutaneous disease.<sup>163</sup> A latent period of many years typically occurs before the patient has constitutional and pulmonary signs and symptoms.<sup>163</sup> It is believed that these occur as a result of reactivation. The radiographic and clinical presentations mimic pulmonary tuberculosis, which may delay diagnosis or treatment.<sup>164</sup> The most common radiographic findings include a relatively symmetric, parahilar coarse interstitial process, often with a prominent nodular component. Pulmonary opacities have a tendency to coalesce and extend into the lower or upper lobes. A second common radiologic appearance is foci of airspace consolidation that may be bilateral and extensive.<sup>165,192,195</sup> Cavitation may occur in areas of consolidation. Upper lobe fibrosis with bulla and cavity formation, bronchiectasis, and pleural thickening are common in more advanced cases.<sup>166</sup> Upper lobe cavitory disease may be associated with lower lobe clustered centimeter-sized nodules, perhaps representing endobronchial disease.<sup>167</sup>

Pulmonary disease is usually a relentless, progressive process without therapy,<sup>168</sup> but rarely resolves spontaneously.<sup>169</sup> Lymphadenopathy and pleural effusion are unusual.<sup>162</sup> Solitary or multiple (0.5 to <2 cm) masses (paracoccidioidomycoma),<sup>170</sup> a solitary, thick-walled cavity in a lower lobe,<sup>171</sup> and miliary nodules are uncommon.<sup>170</sup>

## Pseudallescheriasis

Although *Pseudallescheria boydii* is the most common cause of mycetoma in the United States,<sup>172-174</sup> pulmonary infection is rare.<sup>175</sup> In most circumstances an underlying pulmonary condition acts as a predisposing factor.<sup>174,176</sup> Risk factors for *P. boydii* infections include: chronic fibrotic or fibrocavitary abnormalities,<sup>173,176</sup> atypical mycobacterial infection,<sup>174</sup> bronchiectasis<sup>176</sup> or other disorders, sarcoidosis,<sup>172</sup> ankylosing spondylitis<sup>176</sup> and immune suppression due to malignancy,<sup>177,178</sup> chemotherapy<sup>213,215</sup> corticosteroids,<sup>172</sup> or organ transplantation.<sup>238,244</sup> However, cases



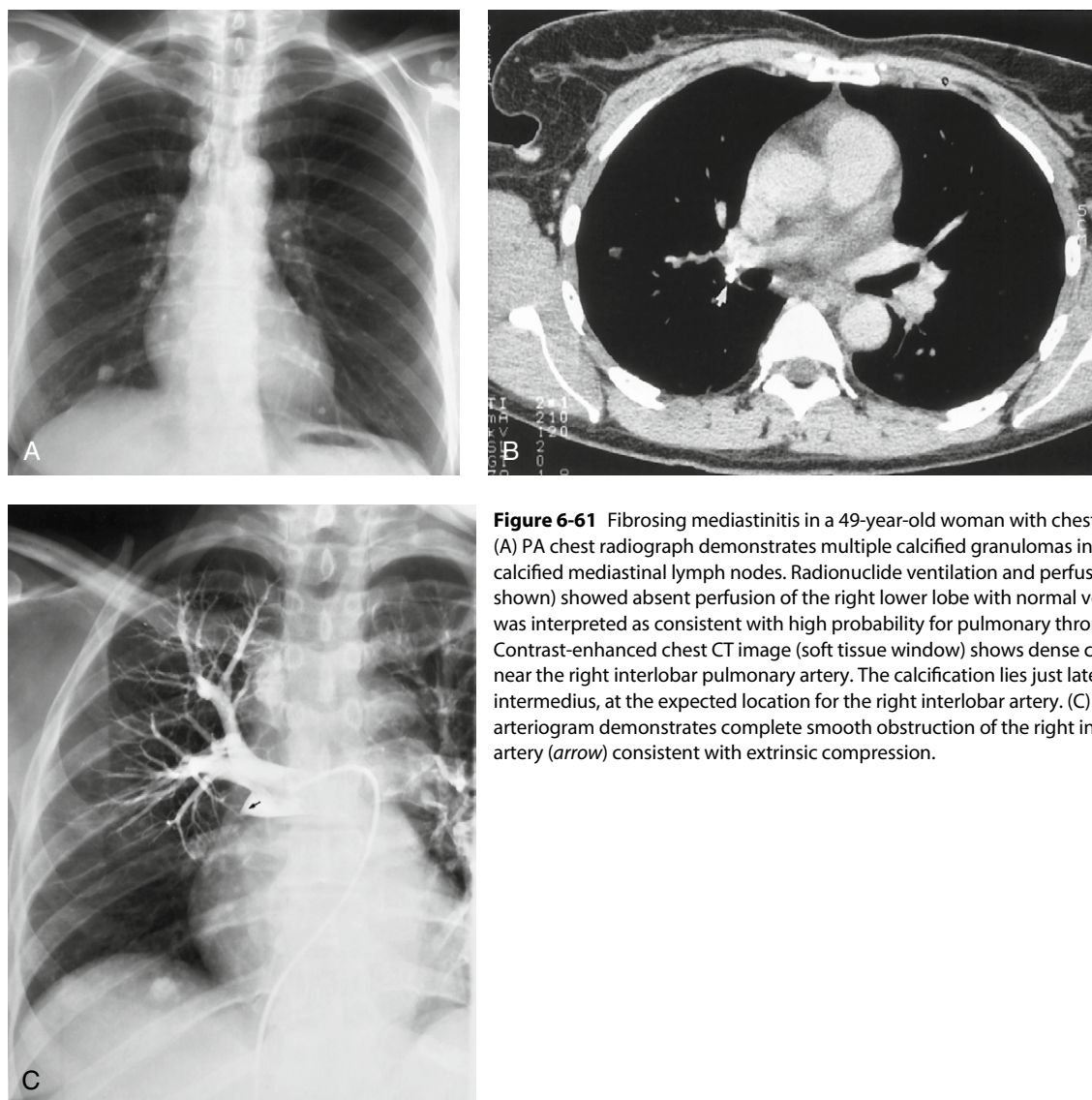


**Figure 6-60** Fibrosing mediastinitis. (A) Coned PA chest radiograph of a 26-year-old woman shows a subtle, large subcarinal mass (*black arrows*) which narrows the transverse diameter of the bronchus intermedius (*white arrow*). The right lung volume is reduced when compared to the left. (B,C) Single posterior images of the right (R) and left (L) lungs from radionuclide ventilation (B) and perfusion (C) lung scans demonstrate reduced lung volume (B) and absent perfusion (C) of the right lung; only the left lung exhibits radionuclide uptake. (D) Axial T1-weighted MR image demonstrates an intermediate signal intensity subcarinal mass (*asterisk*) which obstructs the right interlobar pulmonary artery (*white arrow*) and severely narrows the bronchus intermedius (*black arrows*). (E) Axial T2-weighted MR image through the same level as (D) demonstrates that a large portion of the mass is composed of heterogeneous low signal consistent with fibrous tissue. (F) Coronal T1-weighted MR image demonstrates the large subcarinal mass and the slit-like narrowing of the bronchus intermedius (*arrows*). (Increased signal from within the mass is due to copy artifact.)

with no risk factors and an apparently normal immune system have contracted pulmonary pseudallescheriasis.<sup>199,207</sup>

In many ways, pulmonary pseudallescheriasis is very similar to pulmonary aspergillosis with involvement of the tracheobronchial tree and lungs. Most *P. boydii* isolates from

lung specimens represent colonization.<sup>179</sup> The organism is acquired through inhalation<sup>172,238,244</sup> with subsequent colonization of walls of the bronchi<sup>173,176</sup> or the pulmonary cysts or cavities.<sup>172,179</sup> When only the bronchial walls are colonized, interval thickening occurs. When preexistent pulmonary cysts



**Figure 6-61** Fibrosing mediastinitis in a 49-year-old woman with chest pain for 4 weeks. (A) PA chest radiograph demonstrates multiple calcified granulomas in the lung and calcified mediastinal lymph nodes. Radionuclide ventilation and perfusion lung scans (not shown) showed absent perfusion of the right lower lobe with normal ventilation. The study was interpreted as consistent with high probability for pulmonary thromboembolism. (B) Contrast-enhanced chest CT image (soft tissue window) shows dense calcification (arrow) near the right interlobar pulmonary artery. The calcification lies just lateral to the bronchus intermedius, at the expected location for the right interlobar artery. (C) Right pulmonary arteriogram demonstrates complete smooth obstruction of the right interlobar pulmonary artery (arrow) consistent with extrinsic compression.

or cavities become colonized, the most common manifestation is a fungus ball of *P. boydii*.<sup>172,173,175,176</sup> The radiologic features are identical to those of an *Aspergillus* fungus ball. Uncommonly, multiple *P. boydii* fungus balls develop.<sup>176</sup> In view of the similarities between the two organisms, differentiation often requires culture or serum precipitins.

Less common manifestations include necrotizing pneumonia or focal airspace consolidation.<sup>175</sup> Most patients are immunosuppressed,<sup>177,179</sup> often with leukemia and severe neutropenia. Hung et al described a patient with normal immunity and pneumonia that invaded the pleura, adjacent ribs and spine.<sup>179</sup> Pneumonia may be accompanied by multiple small nodular opacities in the adjacent lung.<sup>177</sup> Other unusual findings reported with *P. boydii* pneumonia include the “halo sign,” crescentic cavitation surrounding a rounded mass.<sup>178,208,209</sup> An unusual manifestation of the disease, a solitary pulmonary nodule representing focal *Pseudallescheria* pulmonary abscess, was reported in two patients, one in a heart transplant recipient<sup>180</sup> and the other in an asymptomatic patient with normal

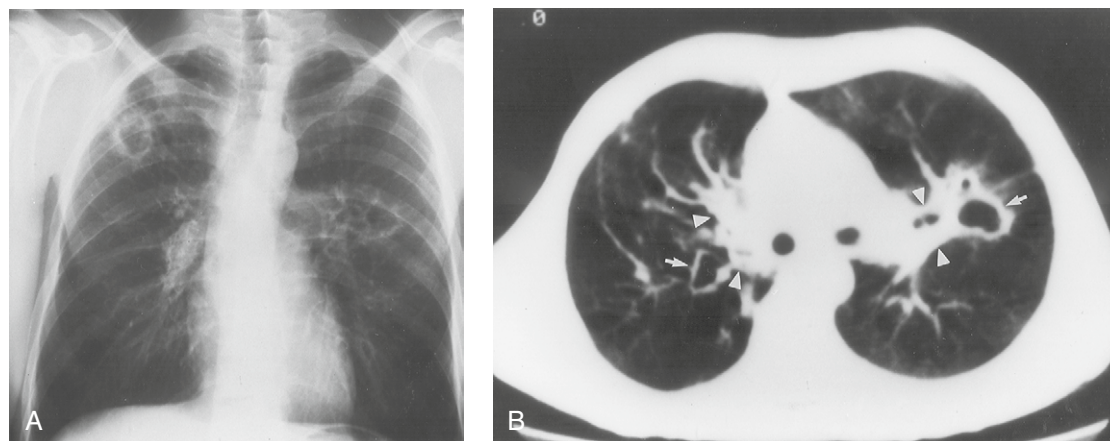
immunity.<sup>181</sup> Also reported are miliary disease and allergic bronchopulmonary pseudallescheriosis (ABPP).<sup>182</sup>

## Sporotrichosis

Pulmonary infection with *Sporothrix schenckii* is rare. Lung disease most commonly results from inhalation of the *S. schenckii* organism.<sup>183</sup> The rarity of primary pulmonary sporotrichosis and the much greater frequency with which postprimary tuberculosis produces identical clinical, pathologic, and radiographic features often prompt prolonged treatment with antituberculous medications and delay correct diagnosis for years.<sup>184</sup> Accordingly, every undiagnosed chronic cavitary pulmonary disease should prompt appropriate tests for sporotrichosis.

The more common form of pulmonary sporotrichosis is a chronically, often relentlessly progressive infection, occasionally despite therapy with antifungal agents and surgical extirpation.<sup>183</sup> Response to therapy may not be immediate and





**Figure 6-62** A 40-year-old white man with known sporotrichosis who had continued symptoms despite treatment with ketoconazole. (A) PA chest radiograph shows fibrocavitary disease involving the right upper lobe and the superior segment of the left lower lobe. A right upper lobectomy was subsequently performed revealing sporotrichosis. (B) Chest CT (lung windows) demonstrates a thin-walled cavity in both upper lobes (arrows). Marked thickening of the interstitium surrounding hilar bronchovascular structures is present bilaterally (arrowheads).

patients with chronic parenchymal involvement may still die.<sup>185</sup> *Chronic upper lobe cavitary disease associated with fibrosis and volume loss is the most frequent radiographic manifestation* (Fig. 6-62). Occasionally cavities are surrounded by airspace and interstitial disease. Cavities are typically several centimeters in diameter. Less common presentations include focal consolidation, a solitary mass or a diffuse, reticulonodular process (Fig. 6-63).<sup>183</sup> In a series of eight patients from the Armed Forces Institute of Pathology,<sup>186</sup> six patients (75%) had pulmonary cavities and in half the cavities were bilateral and apical. Right and left lungs were equally affected. The less common form of pulmonary disease is acute and rapidly progressive. Mediastinal and hilar lymph nodes are affected, usually in the absence of a pulmonary opacity. The lymph nodes enlarge, occasionally causing bronchial obstruction.<sup>187</sup> Pulmonary consolidation, if present, is usually transient. Symptoms resolve spontaneously, leaving enlarged lymph nodes.<sup>187</sup>

## Zygomycosis

Pulmonary zygomycosis typically manifests radiographically as a rapidly progressive parenchymal consolidation that may affect one or more pulmonary lobes (Fig. 6-64). Focal or multifocal pulmonary nodules or masses are the next most frequently seen radiographic manifestation. Cavitation within consolidations, nodules or masses is seen in approximately 40% of patients.<sup>139,222</sup> Pulmonary gangrene may develop within a dense area of consolidation. Cavitation may occur, and a crescentic lucency may develop around the devitalized lung in the dependent portion of the cavity (see Fig. 6-64).<sup>188</sup> However, this “air crescent” sign, which is characteristically seen in patients with angioinvasive fungal infections, particularly invasive aspergillosis, is less frequent. Mediastinal lymphadenopathy and pleural effusion are less frequent.<sup>189</sup>

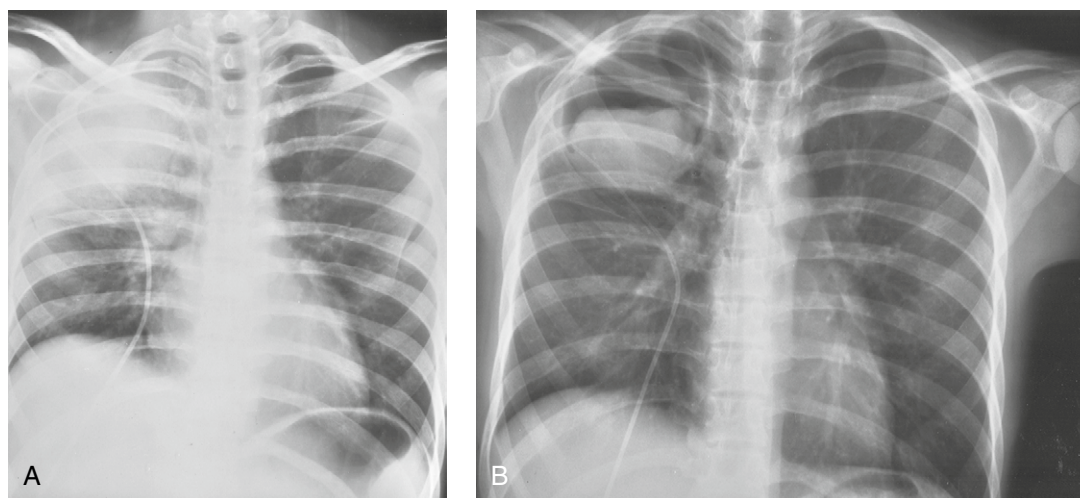
CT may demonstrate significant findings not evident on chest radiography in approximately 26% of cases.<sup>189</sup> Low attenuation of the affected lung parenchyma may be seen in patients with resultant pulmonary infarction. The “CT halo”



**Figure 6-63** Sporotrichosis. AP chest tomographic image of the left lung base demonstrates an oval opacity behind the cardiac apex (arrows).

sign, consisting of a halo of ground-glass opacity around a pulmonary nodule, may be seen in pulmonary zygomycosis.<sup>189</sup> Other manifestations of invasive fungal infection not evident on plain radiography that may be visualized with CT include vascular, mediastinal, bronchial, and upper abdominal involvement.<sup>190</sup> The diagnosis of pulmonary zygomycosis typically requires biopsy. Sputum cultures and cultures of bronchoalveolar lavage and needle aspiration specimens are rarely positive.<sup>189</sup> Radiologists must have a high index of suspicion when





**Figure 6-64** Zygomycosis in an 18-year-old man with acute lymphocytic leukemia who had just completed the first 7-day course of chemotherapy. He complained of increasing lethargy, night sweats, anorexia, weight loss, anemia and leukopenia. (A) PA chest radiograph demonstrates a large area of airspace consolidation in the right upper lobe. Sputum culture grew *Klebsiella* but the patient did not respond well to aggressive antibiotic therapy. Right subclavian vein central line is present. (B) Follow-up PA chest radiograph 2 months later demonstrates a large right upper lobe cavity with a dependent ovoid mass. Bronchoscopy recovered *Rhizopus* species.

evaluating individuals at risk who have manifestations of progressive severe, necrotizing, or multifocal pulmonary disease. Early biopsy and diagnosis may decrease mortality by allowing aggressive surgical and therapeutic intervention.<sup>189</sup>

## Musculoskeletal fungal infections

### Aspergillosis

Although *Aspergillus* infection of the bones is rare, the spine is the site most commonly involved.<sup>191,192</sup> The infection is usually preceded by surgery or instrumentation.<sup>201</sup> Rib involvement is common in children with thoracic spine involvement. Tibia, ileum, wrist, pelvis, ribs, and sternum are the common sites. There are only isolated cases of osteomyelitis where radiographic studies are obtained.<sup>193</sup> The lesions are ill defined with poor margination on plain radiographs. CT scans may demonstrate an area of lucency or the peripheral sclerosis may obscure the central lucency as in the case reported by Sonin.<sup>193</sup> MR imaging can show the marrow edema not evident on the radiographs or CT images (Fig. 6-65). Arthritis due to aspergillosis is very rare and results from hematogenous spread from the lungs in an immunocompromised host. In immunocompetent patients, arthritis or osteomyelitis usually occurs after surgery. Radiographic findings in arthritis are similar to bacterial septic arthritis with joint effusion, synovitis and bone destruction.

### Blastomycosis

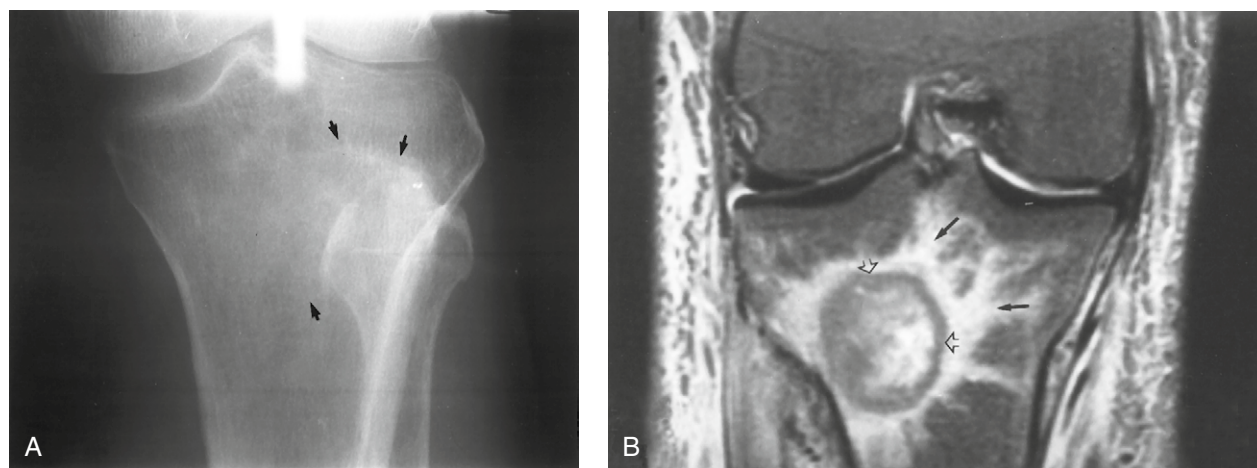
Musculoskeletal involvement is more often seen in those who are active outside such as farmers, hunters, etc. Osteomyelitis is present in 25–50% of cases of disseminated blastomycosis. Bone lesions are more often seen in elderly patients.<sup>194</sup> Vertebrae, pelvis, skull, ribs and long bones are commonly involved.

Bone pain and soft tissue swelling at the site of infection are the common symptoms. The presentation of blastomycosis radiographically is non-specific and often mistaken for a neoplasm.<sup>195</sup> *Blastomyces* osteomyelitis causes lytic lesions which are well circumscribed, eccentric, with little periosteal reaction.<sup>194</sup> *Within the long bones, the metaphysis is most commonly affected.* In flat bones such as the sternum, pelvis and sacrum, extensive erosions can lead to disappearance of the bone (Fig 6-66A). *Sequestration is rare which distinguishes Blastomyces osteomyelitis from chronic bacterial osteomyelitis.* Adjacent soft tissue swelling, subcutaneous abscesses and draining sinuses may be present. The skeletal changes are not unique and correlation with other findings such as pulmonary disease may help in the diagnosis. When bone lesions are asymptomatic, adjacent skin lesions should be looked for as these may suggest the possibility of *Blastomyces* osteomyelitis.

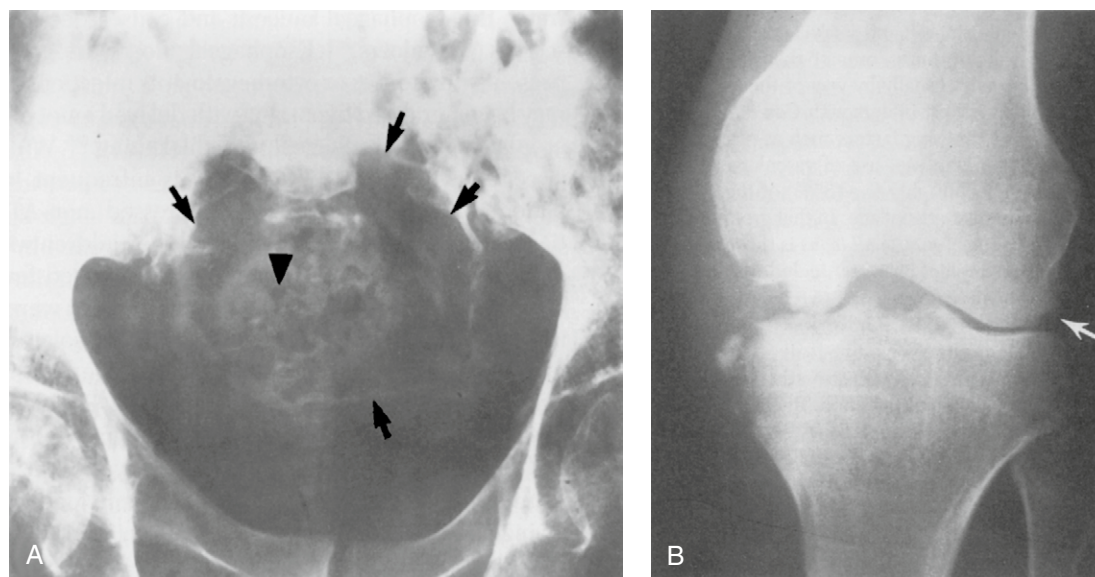
Blastomycosis arthritis is reported but it is rarely the only manifestation. When present, it is typically monoarticular, commonly involving knee, ankle, or elbow. The synovium, ligaments, bones and surrounding soft tissues are usually involved.

### Candidiasis

Bone infection by *Candida* is rare.<sup>196</sup> Infants, IV drug abusers, and HIV-positive patients are at risk for *Candida* arthritis. In IV drug abusers, there is predilection for fibrocartilaginous joints: costochondral, intervertebral and sacroiliac joints. Monoarticular disease is slightly more common than polyarticular disease. Arthritis occurs usually via the hematogenous route in infants. Most infants with *Candida* arthritis have underlying predisposing factors such as prematurity, broad-spectrum antibiotic use, use of vascular catheters, or hyperalimentation.<sup>196</sup> Eighty five percent of affected infants are less than 6 months of age. In this group, in 80% of cases of



**Figure 6-65** *Aspergillus* osteomyelitis in a 60-year-old man with knee pain and history of renal transplant. (A) AP radiograph of the knee demonstrates an amorphous area of sclerosis without a definite lucency in the tibial metaphysis (arrows). (B) Coronal turbo short inversion recovery image (T2WI) shows a well-defined intermediate-intensity lesion with peripheral low intensity (open arrow). This low intensity corresponds to the sclerosis seen in the radiograph. Note the high-intensity marrow edema surrounding the lesion (long arrow). There is reversal of right and left on this image. (Reproduced with permission from Sonin et al.<sup>193</sup>).

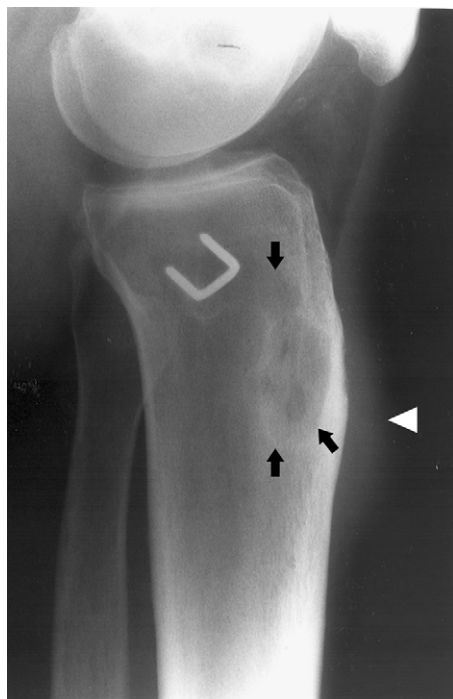


**Figure 6-66** (A) Extensive osteolysis (bone destruction) in blastomycosis. An AP radiograph of the pelvis in an elderly man shows complete destruction of the sacrum, leaving only a thin rim of bone (arrows). There is superimposition of rectal stool (arrowhead). Osteolysis is seen in flat bones involved with *Blastomyces* osteomyelitis. (Case courtesy of Dr Rebecca Lored, San Antonio, TX.) (B) Septic arthritis due to *Candida*. There is joint space narrowing with marginal erosions (white arrow). Also note sclerosis and fragmentation of the distal femur and proximal tibia. (Reproduced with permission from Resnik<sup>202</sup>).

arthritis, *Candida albicans* is the implicated organism. Radiographic changes include soft tissue swelling, joint space narrowing, and irregularity of subchondral bone (Fig. 6.66-B). Unlike bacterial arthritis, bone destruction is not excessive. *Candida* osteomyelitis is a late manifestation of hematogenous dissemination.<sup>197</sup> The site of osteomyelitis varies with age. The spine is most commonly involved in adults<sup>198</sup> and the long bones in children.<sup>199</sup>

## Coccidioidomycosis

Approximately 50% of patients with dissemination have skeletal lesions.<sup>200</sup> The most commonly involved areas are spine, pelvis, hands and lower extremities. When the spine is involved there is narrowing of the disk space with erosive changes of the end-plates similar to other infections of the spine. Radiographs frequently show multiple osseous lesions.



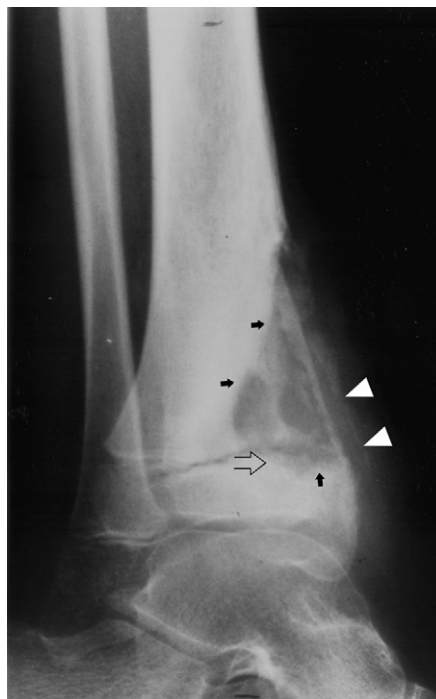
**Figure 6-67** *Coccidioides* osteomyelitis in a 40-year-old man. Lateral radiograph shows a well-defined lucency without a sclerotic margin (arrow) in the tibial metaphysis. Note the adjacent soft tissue swelling (arrowheads). The metallic clip was related to prior surgery following trauma. (Case courtesy of Dr Rebecca Lored, San Antonio, TX.)

On radiographs, the lesions are lytic with well-defined margins in the metaphysis. Sclerosis of the margins is not common. Involvement of bony prominences such as tibial tuberosity and adjacent soft tissue thickening is common (Fig. 6-67). *Well-demarcated lytic areas without sclerotic margins are typical of Coccidioides infection. Sequestration is unusual*, unlike chronic osteomyelitis due to bacterial infections. There may be periosteal new bone formation and the lesion may mimic an aggressive tumor (Fig. 6-68). Radionuclide bone scan studies are useful in demonstrating multiple sites of involvement (Fig. 6-69).

An acute arthritis with pain and swelling of the joints is reported in one-third of cases. However, in 10–20% of these cases, bone or joint changes are seen. The ankle and knee are the two joints most commonly involved. Most often, only one joint is involved.<sup>201</sup> Small effusions are common. The inflammation starts in the synovium and extends into the cartilage and the underlying bone. Radiographic evidence of bone destruction is usually absent in the early course of joint infection, presumably due to the low virulence of the pathogen. MRI shows subchondral edema and enhancement, which are likely reactive.<sup>201</sup> Joint space narrowing, osteopenia and bone destruction are commonly seen later in the course (Fig. 6-70). Involvement of bursa and tendons, causing bursitis and tenosynovitis of the hand and wrist, is reported.<sup>202</sup>

### Cryptococcosis

In 5–10% of cases of disseminated cryptococcal infection, osseous involvement is seen and is usually associated with disseminated disease.<sup>203</sup> Most commonly spine, pelvis, ribs, skull



**Figure 6-68** Aggressive *Coccidioides* lesion mimicking a tumor in a 12-year-old who presented with joint pains. An oblique view of the ankle demonstrates a lytic lesion in the distal tibia (arrows) with periosteal bone formation (white arrowheads). Note that the lesion crosses the physis (growth plate) into the epiphysis (open arrow). Soft tissue swelling is also present. There was right elbow involvement on other radiographs (not shown). (Case courtesy of Dr Rebecca Lored, San Antonio, TX.)

and tibia are involved. Bony prominences such as the tibial tuberosity and the femoral trochanter may be affected. The lesions are osteolytic with discrete margins with little or no periosteal reaction (Fig. 6-71). *Periosteal new bone formation is limited in cryptococcosis*. Most lesions demonstrate little change in radiographic appearance over time.<sup>203</sup>

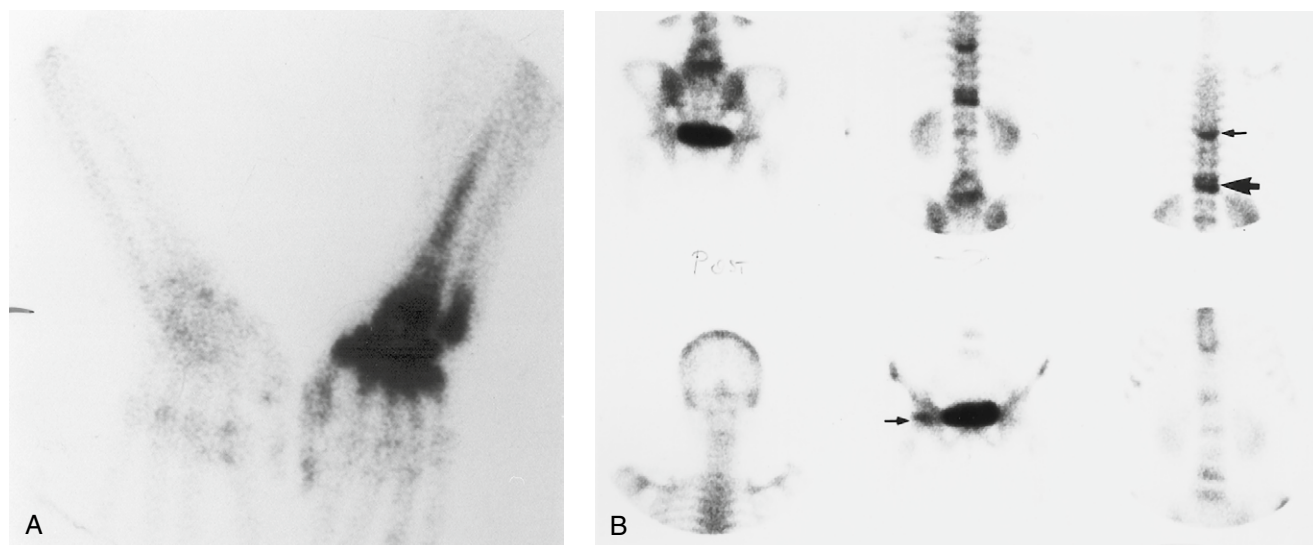
### Histoplasmosis

Skeletal involvement is more common with *H. capsulatum* var. *duboisii* than *H. capsulatum*. Pelvis, skull, ribs and small tubular bones are frequently involved.<sup>202</sup> Children are more commonly involved than the adults. The lesions are seen as areas of lucency of variable size with well-defined margins. When the lesions involve the diaphysis, there may be extensive periosteal new bone formation along the outer surface of the bony cortex (Fig. 6-72A). In cases of *H. capsulatum* var. *duboisii* the bone lesions are accompanied by skin lesions in 80% of cases. Cystic lytic bones are more characteristic in *H. capsulatum* var. *duboisii* infection (Fig. 6-72B). Arthritis due to histoplasmosis is rare.<sup>204</sup>

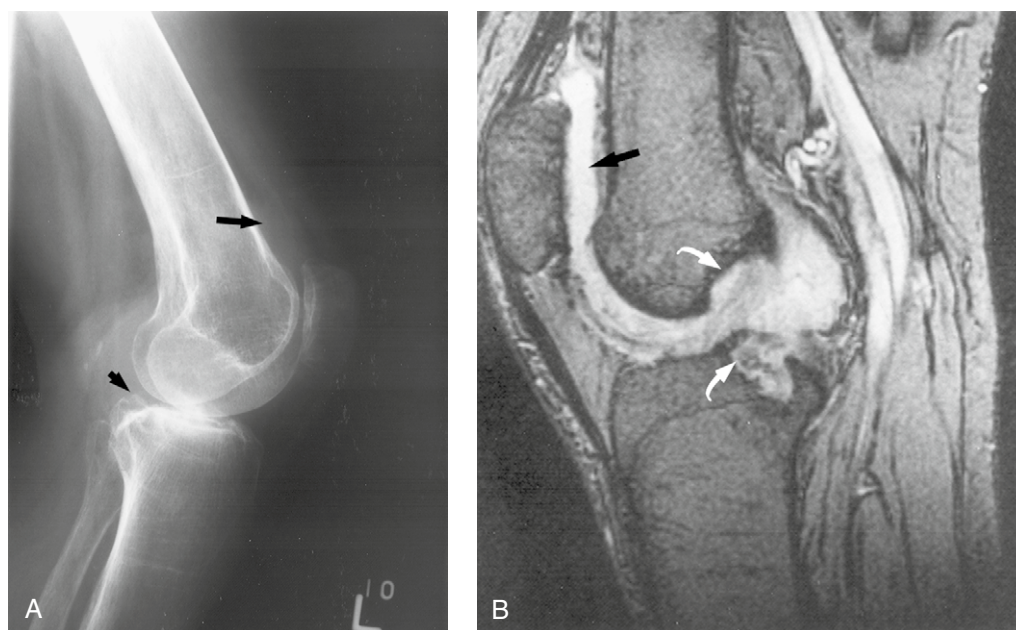
### Paracoccidioidomycosis

This is rarely seen in the United States. The lesions are usually centered in the bone and manifest radiographically as circumscribed lytic lesions with or without a rim of sclerosis.<sup>205</sup> Tubular and flat bones are involved. The radiographic changes





**Figure 6-69** Multiple “hot spots” due to *Coccidioides* on radionuclide bone scans in a 60-year-old oriental woman who presented with left wrist pain. (A) A spot image from a bone scan using Tc 99m methylene diphosphonate (MDP) demonstrates marked increased activity in the left wrist. (B) Spot images demonstrate other “hot spots” in the thoracolumbar vertebra and right femoral head (arrows). Increased uptake in the both sacroiliac joints is normal. (Case courtesy of Dr Ralph Blumhardt, San Antonio, TX.)



**Figure 60-70** Chronic *Coccidioides* arthritis. (A) Lateral view of the left knee shows osteopenia, joint effusion (arrow) and an area of bone erosion (small arrow). (B) MR imaging of the knee (T2WI) demonstrated large areas of erosions posteriorly (curved arrow) as well as the joint effusion (arrow).

of joint involvement are non-specific and include joint effusion, subchondral erosions, and narrowing of the joint space.<sup>205</sup> Overall, the radiographic changes are similar to those of the other fungal infections.

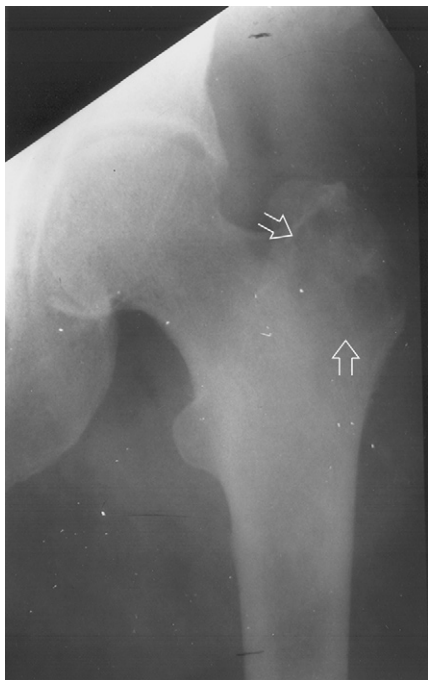
### Pseudallescheriasis

Infection with *Pseudallescheria boydii* most often results in the clinical syndrome of mycetoma which usually involves the foot—Madura foot.<sup>206</sup> Maduramycosis is a chronic granulomatous

infection of the foot, typically seen in tropical and subtropical regions of the world. In the United States there are several causative organisms but *P. boydii* is the most frequent cause of Madura foot. It can also involve the hands, arms, legs, and scalp after a soft tissue injury with invasion by the organisms.

Initially patients present because of soft tissue swelling. In chronic cases, there may be cutaneous sinuses discharging fungal elements. Radiographs of the involved area may show extensive sclerosis (increased density) with periosteal new bone formation (linear bone formation along the outer cortex)

(Fig. 6-73). Bony lucencies due to cavities filled with fungal elements may also be seen. Several bones and joints in an area may be involved, mimicking the neuropathic joints. There may be secondary bacterial infection. Sharif et al<sup>207</sup> studied the CT and MR imaging findings in 18 patients and found that they



**Figure 6-71** *Cryptococcus* osteomyelitis. AP radiograph of the left hip shows a poorly defined round lucency in the greater trochanter of the femur (open arrows). There is no marginal sclerosis or periosteal new bone. (Case courtesy of Dr Vung Nguen, San Antonio, TX.)

were comparable and that CT was adequate in these cases. CT studies demonstrated cortical thickening, periosteal new bone formation along the cortex and soft tissue edema with diffuse enhancement of the soft tissues involved. On MR imaging, the involved tissues had low signal intensity on T1 with moderate increased signal on T2 images. Gadolinium enhancement did not provide any additional information compared to the T1 images. Soft tissue involvement was better noted on the T1WI compared to T2 images.<sup>207</sup>

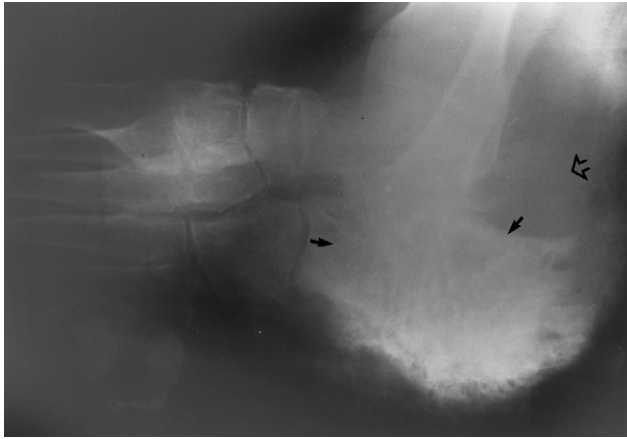
## Sporotrichosis

This may be related to a local wound from which the infection extended into the joints or bones or be due to hematogenous infection. Thus people who work outside such as farmers, gardeners or florists are more prone to this infection. Bone and joint involvement occurs by contiguous spread of a cutaneous foci or hematogenous dissemination.<sup>208</sup> Arthritis is extremely unusual but when present, radiographs are abnormal in 92% of cases.<sup>208</sup> The joints most commonly involved are knee (64%), hand and wrist (50%), elbow (24%), and ankle (20%). Articular involvement can be mono-, oligo- or polyarticular. There is soft tissue swelling without much joint space narrowing. Irregularity and poor definition of the articular ends may be seen in early cases. With progression, there may be extensive bone destruction mimicking bacterial septic arthritis (Fig. 6-74). Extremity bones such as tibia, fibula, humerus and small bones of the hand are frequently involved. When the bone involvement is secondary to a local wound the lesions appear as eccentric erosions adjacent to the subcutaneous lesion. Multiple punched-out lytic lesions are seen with hematogenous spread of the infection. *Osteolysis predominates with little or no periosteal reaction*. Direct involvement of joints without bone involvement is typical of sporotrichosis.



**Figure 6-72** (A) *Histoplasma* osteomyelitis of the tibia. Lateral radiograph shows a large lytic lesion in the tibial diaphysis with well-defined margins. Note the periosteal new bone formation. (With permission from reference 202.) (B) Histoplasmosis cystic bone lesions. Frontal radiograph of the left hand reveals multiple well-marginated cystic lesions due to *Histoplasma capsulatum* var. *duboisii*. (Reproduced with permission from Resnik<sup>202</sup>).





**Figure 6-73** Mycetoma of the foot in a patient with chronic soft tissue swelling and draining sinuses. Lateral radiograph shows marked sclerosis and irregularity of the calcaneus (arrows). Although confined to a single bone in this case, mycetoma may involve several bones. A joint effusion is also noted (open arrow). When there are draining sinuses the causative fungal organism can be recovered from the discharged contents. (Case courtesy of Dr Rajendra Kumar, Houston, TX.)



**Figure 6-74** Sporotrichosis elbow arthritis. There is soft tissue swelling with marked bone destruction involving the distal humerus, proximal radius and ulna. When there is significant bone loss this may mimic bacterial arthritis. (Reproduced with permission from Resnik<sup>202</sup>).

## Abdominal mycotic infections

### Aspergillosis

Abdominal disease is rare with a few case reports in the literature.<sup>213-217</sup> Most patients with abdominal involvement are immunocompromised or have undergone organ

transplantation. Although at autopsy, the GI tract, liver and kidneys are involved, only a few reports have described the imaging findings.

Disseminated aspergillosis can cause hemorrhagic infarcts in abdominal organs due to blockage of the end vessels by the fungal mycelium. Sonography and CT are most useful in evaluating such patients. Frank abscesses are not common due to the poor phagocytic function of the macrophages in immunocompromised patients. Abscesses appear as cystic regions on sonography compared to poorly formed foci of inflammation or infarcts which are hypoechoic. A central hyperechoic focus may be seen in the center of the lesion if infarction has occurred. Similarly, on CT these appear as hypodense areas. A central hyperdensity indicates infarction (Fig. 6-75). These findings are not unique to aspergillosis, as similar findings were reported in a leukemic patient with zygomycosis of the liver.<sup>221</sup> Isolated cases of mycotic aneurysms or abdominal surgical wound infections are reported.<sup>209</sup>

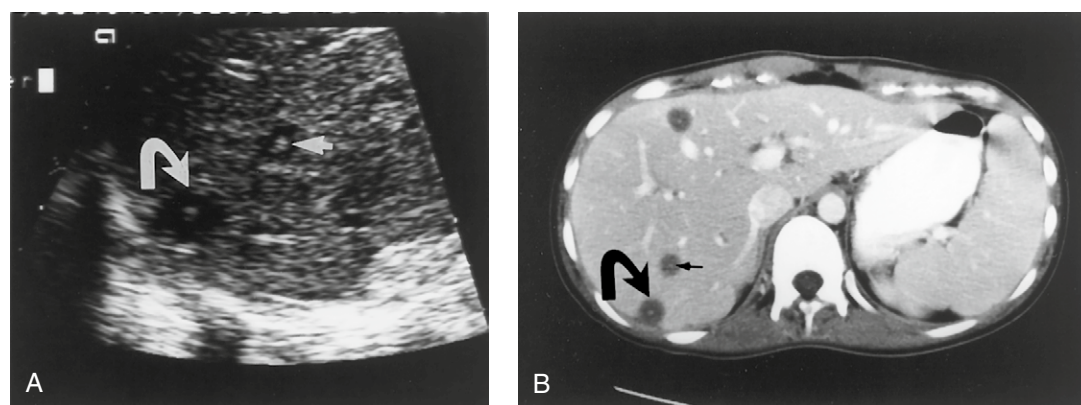
Disseminated aspergillosis with gastrointestinal invasion is a rare and highly lethal opportunistic infection that usually involves neutropenic patients. Trésallet et al<sup>210</sup> reported a case of small bowel infarction and obstruction with a pathologically confirmed invasive aspergillosis. CT showed a diffuse small bowel dilatation with wall thickening of the ileum. At pathology, small bowel ulcerations with necrosis were seen.

Urinary tract involvement is rare. Kay<sup>211</sup> described a case of renal aspergilloma in an AIDS patient. The renal lesions were hypoechoic on sonography (Fig. 6-76), like the hepatic lesions. Sonography may demonstrate hydronephrosis due to obstruction. Bibbler and Gianis<sup>212</sup> reported a 34-year-old diabetic with acute flank pain due to ureteropelvic junction obstruction from a fungus ball. A fungus ball is formed by tangled fungal elements with debris or necrosis. When they enlarge, they can cause obstruction to the collecting system. The fungal ball is seen as a filling defect with proximal dilatation of the collecting system on intravenous urogram. On CT the fungus ball is seen as a soft tissue mass in the collecting system. *Aspergillosis and zygomycosis are two opportunistic infections that usually cause infarctions*, as reported by Libshitz and Pagani.<sup>117</sup>

### Blastomycosis

The genitourinary tract is involved in approximately 20–25% of cases.<sup>194</sup> The epididymis and prostate are involved in the majority of cases. On sonography, the enlarged epididymis is hypoechoic. Similar changes may be noted in the prostate gland on sonography. These changes are not specific for blastomycosis and can be seen with any bacterial infections. When chronic prostatitis is suspected, prostatic secretions should be cultured. GI tract involvement is rare with blastomycosis.<sup>213</sup> Cholangitis caused by *Blastomyces* was reported in a non-HIV patient.<sup>214</sup> CT of the abdomen demonstrated dilated bile ducts in the left lobe of liver. Liver biopsy and bile cultures confirmed blastomycosis.<sup>214</sup> Chest radiograph of this patient was normal. Two reports, one of splenic abscess and the other of adrenal glands secondary to blastomycosis, are also described.<sup>215</sup> *In subjects with chronic prostatitis not responding to antibiotic treatment, Blastomyces infection should be considered.*





**Figure 6-75** *Aspergillus* liver abscesses in a young woman with leukemia who presented with abnormal liver function tests and fever. (A) Abdominal sonogram transverse scan through the liver shows hypoechoic areas (curved arrow) with a central echogenic focus (small arrow). (B) CT image of the liver in the same patient shows multiple hypodensities (curved arrow) with central hyperdensities (small arrow). The hyperdensity frequently consists of fungal organisms and necrotic tissue.

## Candidiasis

The gastrointestinal tract is frequently involved by *Candida* infection. Phelan et al<sup>216</sup> reported oral candidiasis in 92 of 103 HIV patients (91%) consecutively seen by them. Odynophagia and dysphagia are two common symptoms of esophageal disease. Double-contrast barium studies of the esophagus characteristically show discrete “plaque-like” defects with intervening normal mucosa. With diffuse involvement, the esophagus has a diffuse, characteristic “shaggy” appearance (Fig. 6-77). This is due to the barium trapping under the pseudomembranes formed by the *Candida*. In severe cases, there may be ulceration, although it is uncommon. The yeast form produces surface granulomas and membranes, while the hyphal form penetrates the esophageal mucosa and causes deep microabscesses and ulcers. Esophageal ulcers are more often present with herpes or cytomegalovirus infection.<sup>217</sup> There may be generalized hypotonia with delayed emptying of the esophagus and esophageal wall thickening. *Widespread visceral involvement by Candida* is infrequent in AIDS patients unlike the immunosuppressed non-AIDS patients.<sup>218</sup> In a study by Bartley,<sup>219</sup> all the children with *Candida* infection had neutropenia and prolonged fevers not responding to antibiotics. Liver and spleen are usually involved; renal infection was uncommon. *Isolated spleen involvement was uncommon.*

Typically, the lesions in the liver and spleen are multiple and small. Pastakia et al<sup>220</sup> have reported four patterns on sonography:

1. “wheels within wheels” appearance
2. bull’s eye lesion
3. uniform hypoechoic lesion
4. echogenic shadowing foci.

The uniform hypoechoic pattern is more frequent than the others. The “wheels within wheels” is seen early in the infection. There is a hypoechoic outer ring due to fibrosis with an echogenic inner ring from inflammatory reaction. In the center of the inner wheel, there is a tiny hypoechoic nidus. The central nidus contained fungal elements and necrotic tissue. On CT scans, these areas are poorly defined, with no or minimal peripheral enhancement (Fig. 6-78). They differ from

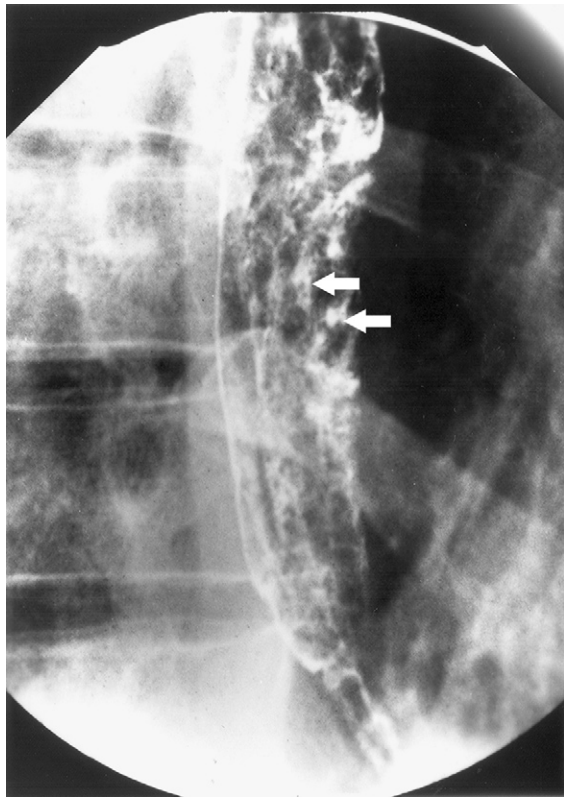


**Figure 6-76** Renal aspergillosis in an AIDS patient. Sonogram shows multiple, round, hypoechoic renal lesions (arrows) in the cortex extending into the corticomedullary junction. (Reproduced with permission from Kay<sup>211</sup>).

both bacterial and amebic abscesses by their size, number, and significantly less or minimal inflammatory changes.

Biliary dilatation and *Candida* fungus balls in the dilated ducts have been described.<sup>221,222</sup> On percutaneous transhepatic cholangiography, the dilated biliary tree had filling defects due to fungus balls.<sup>221</sup> On sonography, dilated ducts are seen as tubular structures accompanying the portal venous radicals. Johnson et al<sup>215</sup> noted that CT was superior to sonography or scintigraphy in detecting hepatosplenic lesions. Semelka et al<sup>223</sup> reported that MR imaging is better than CT in evaluating abdominal candidiasis. Gadolinium-enhanced MR imaging detected lesions not seen by CT or T1 and T2 images. Of the 106 lesions seen on contrast-enhanced MR images, only 20 lesions were seen on T2-weighted images and 18 on CT. T1-weighted (FLASH) sequence demonstrated 85 lesions. These microabscesses have low signal on T1 and high signal

on T2 images. In an earlier report DeGregorio et al<sup>224</sup> noted that the diagnosis of visceral candidiasis was made in only nine of 32 cases before death. With the frequent use of imaging techniques, lesions are more often seen. *When there is severe neutropenia the visceral lesions may be subtle and only seen well after recovery of neutropenia.* Percutaneous needle biopsy of the lesions is necessary to find the organism as the imaging findings are non-specific. Due to chronic indwelling catheters



**Figure 6-77** Severe candidiasis of esophagus in a 29-year-old HIV-positive man who presented with dysphagia. There is marked irregularity of the esophageal lumen due to diffuse plaque-like lesions. Barium trapping under the pseudomembranes (arrows) gives the so-called “shaggy” appearance.

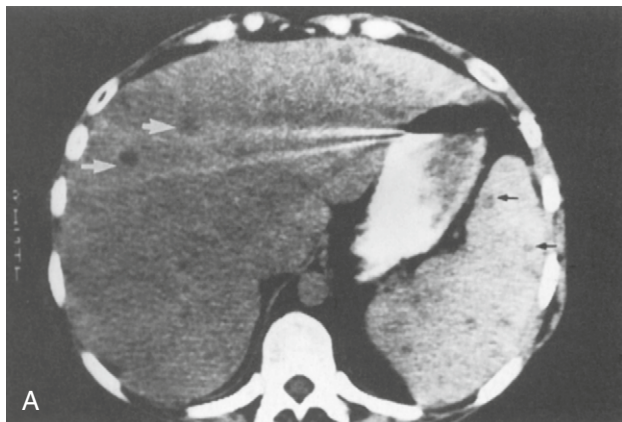
in diabetics on peritoneal dialysis, peritoneal infection by *Candida* occurs in this subgroup of patients.<sup>225</sup> There may be peritoneal fluid on sonography or CT. Aspiration and culture of peritoneal fluid is needed to confirm the diagnosis.

Candidiasis of the urinary bladder is frequent in patients with catheters or ureteral stents. *It is the most common cause of fungal infections of the bladder.*<sup>226,227</sup> Involvement of the kidneys and ureter is seen in patients with obstructive uropathy, malnutrition and prolonged antibiotic use.<sup>235</sup> Candiduria may be the first manifestation of disseminated disease. An excretory urogram (IVP) may demonstrate hydronephrosis, a focal mass or a non-functioning kidney. On imaging studies, focal areas of segmental hypodensities are present in cases of pyelonephritis. These are seen as hypoechoic regions when detected by sonography. In patients with compromised renal function, sonography is useful for evaluating kidneys. There is separation of the central renal echoes when hydronephrosis is present. If the hydronephrosis is significant, dilated calices entering into the dilated renal pelvis may be seen.

CT is superior to excretory urography and sonography. Whenever there is hydronephrosis, CT evaluation of the kidneys may be done to exclude pyelonephritis or perinephric abscess not evident on excretory urography or sonography. CT will demonstrate the perinephric fluid and air collections in cases of perinephric abscess. In some cases, hydronephrosis was due to a fungus ball (Fig. 6-79). Ureteral stent or percutaneous nephrostomy tube placement may be needed to relieve the obstruction (Fig. 6-80). In the 1980s there was an increased incidence of infections due to other species such as *Torulopsis*.<sup>227</sup>

## Coccidioidomycosis

Abdominal disease is rare, although at autopsy liver and spleen are involved.<sup>228</sup> Four of seven patients included in this series were immunocompromised. *In all the patients the chest radiographs were abnormal.* In one of our patients with disseminated disease, hypodense lesions were seen in the spleen, in addition to vertebral and psoas muscle involvement (Fig. 6-81). Biopsy of the mass was done to confirm the diagnosis.<sup>229</sup> Involvement

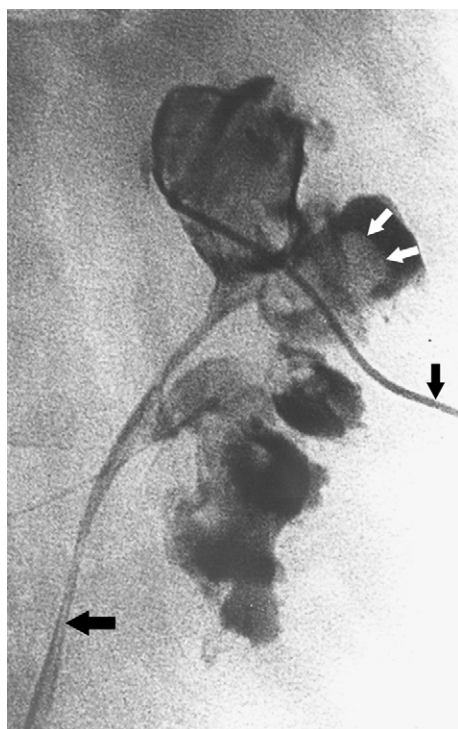


**Figure 6-78** *Candida* hepatic, splenic and renal abscesses in an immunosuppressed leukemic patient. (A) Non-enhanced CT scan through the upper abdomen demonstrates multiple small, round hypodensities (arrows) in the liver and spleen. (B) Enhanced CT scan shows low-density lesions (arrows) in the liver and right kidney. (Case courtesy of Dr Isaac Francis, Ann Arbor, MI.)



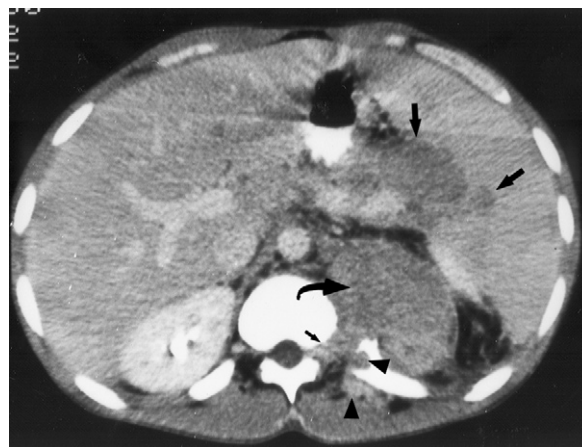


**Figure 6-79** Hydronephrosis due to a *Candida* fungus ball. Longitudinal sonogram of the right kidney shows dilated calyces (arrowheads) joining a dilated renal pelvis (curved arrow). There is an echogenic (dense) focus (arrow) which represents the fungus ball made of fungal elements and necrotic debris. (Reproduced with permission from Kay<sup>211</sup>).



**Figure 6-80** Drainage of hydronephrosis due to *Torulopsis*. In this patient with hydronephrosis on sonography (not shown), a percutaneous nephrostomy tube and ureteral stent were placed (arrows). Note filling defects (white arrows) in the dilated calyces due to debris and fungal elements.

of other organs such as peritoneum, ovaries, adrenals, genitourinary and biliary tract was described. Genitourinary tract infection occurs in cases of dissemination. In autopsy studies, renal (50%), prostate, epididymis and tuboovarian involvement is reported. Genitourinary tract infection occurs in cases of dissemination.



**Figure 6-81** Splenic and psoas lesions in a man with disseminated *Coccidioides* infection. Two hypodense lesions without peripheral enhancement are seen in the spleen (arrows). The paravertebral mass in the psoas muscle (curved arrow) shows some enhancement. The mass extends into the intervertebral foramina (small arrow), and along the posterior aspect of the adjacent rib which is destroyed (arrowheads).

Sohail et al<sup>230</sup> reported on 30 men (median 58 years old) who had coccidioidomycosis of the genital tract, including six at their own institution. Four patients (13%) had a simultaneous pulmonary infection and 19 patients (63%) had a remote history of primary pulmonary coccidioidomycosis. The most commonly involved genital tract sites were the epididymis in 18 cases, prostate in 14 and testes in six. Patients with prostatitis presented with urinary obstruction, with tender nodular enlarged prostate. *Most patients with epididymal infection presented with scrotal swelling, tenderness and induration.* All 30 patients (100%) had histopathologic evidence of granulomatous inflammation with fungal spherules. Urine fungal cultures were positive in 19 cases. Peritoneal involvement resembling diffuse peritoneal carcinomatosis was reported by Eyer et al<sup>231</sup> in a patient with meningitis. In women the involvement of ovaries can resemble ovarian cancer with peritoneal spread. Biliary tract involvement can present as obstructive jaundice with irregular strictures. Extensive involvement of the adrenals has been noted at autopsy in 17–36% of cases with dissemination.

## Cryptococcosis

Abdominal involvement is rare with isolated case reports.<sup>232,233</sup> Hepatitis and biliary obstruction were reported. Imaging features of hepatitis are variable with hepatomegaly or decreased liver echogenicity. Biliary dilatation with or without strictures and wall thickening was seen with cholangitis.<sup>234</sup> Culture of the bile and liver biopsy confirmed cryptococcosis. Sonography and CT are helpful in identifying the biliary dilatation and in excluding a mass. Involvement of the GI tract at autopsy is reported but is rare.<sup>232</sup> In the same report of 41 cases, kidney was involved in 20 and prostate in six at autopsy. The renal lesions on excretory urogram (IVP) were located in the cortex with mass effect on the adjacent collecting system. In one case, there was abnormal contrast collection in the renal papilla due to papillary necrosis. Rarely, cryptococcosis can present with intraabdominal (mesenteric and retroperitoneal)





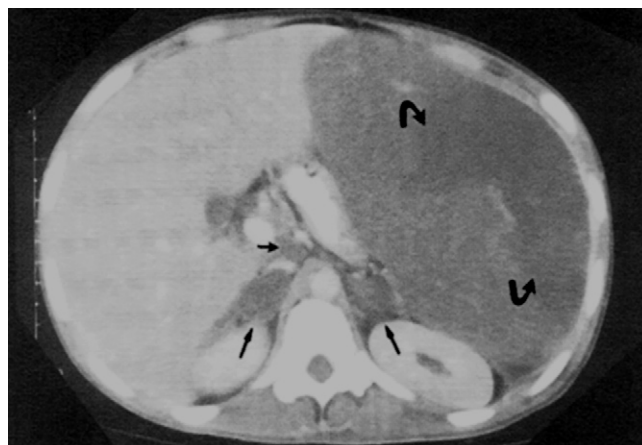
**Figure 6-82** Healed *Histoplasma* granuloma in liver and spleen. Incidental note is made of multiple small calcifications in the upper abdomen (arrows) confirmed to be in liver and spleen by sonography (not shown).

lymphadenitis. Genital infections with *Cryptococcus neoformans* are rare. Ranganathan et al<sup>235</sup> described CT and MRI findings in vaginal cryptococcosis, with a nodular mass in the vagina wall.

## Histoplasmosis

When there is dissemination, liver and spleen are commonly involved.<sup>149</sup> This occurs more often as an acute progressive infection especially in the very young, the very old or those with impaired cellular immunity. When the patients are immunocompromised, they are usually symptomatic. There is hepatosplenomegaly and generalized lymphadenopathy. At this time the sonography of the abdomen may only show hepatosplenomegaly. With healing, the granulomata calcify and are often visible in the liver and spleen on abdominal radiographs (Fig. 6-82). Some times, there may be calcified lymph nodes of 1–3 cm in the splenic and hepatic hila as well. Radin<sup>236</sup> described abdominal CT findings in 16 patients with disseminated histoplasmosis. Fourteen of 16 patients in this report were HIV positive. Hepatomegaly (63%) was seen more often than splenomegaly (38%). Focal hypodense hepatic lesions are not common. When present, these are small (<1 cm) and multiple, unlike larger lesions seen with lymphoma.<sup>245</sup> Three of six patients with splenic disease demonstrated diffuse decreased density of the spleen (Fig. 6-83). Lymphadenopathy involving mesentery, retroperitoneum and porta hepatis was seen in 12 of 16 (75%) patients with disseminated histoplasmosis reviewed by Radin.<sup>236</sup> The lymph nodes were 1–2 cm in diameter with diffuse or central low density, similar to lymph nodes seen in tuberculous infection.<sup>236</sup>

Histoplasmosis may involve the GI tract, especially the terminal ileum and cecum. Gastrointestinal disease is probably a consequence of hematogenous seeding of Peyer's patches. GI tract involvement was present in patients with acute and subacute forms of histoplasmosis but not the chronic form.<sup>246</sup> Autopsy studies have showed ulcers or raised plaques. Later, the ulcerated plaques involve muscularis, leading to perforation in some cases. Mucosal lesions can also coalesce and form circumferential lesions. In HIV-positive patients, the colon appears to be the most commonly involved site, whereas in



**Figure 6-83** Histoplasmosis of the spleen, adrenal and lymph nodes. There is marked splenomegaly with decreased density due to histoplasmosis. Peripheral lower density is due to infarct in the spleen (curved arrows). Note enlarged adrenals (long arrows) and lymph nodes (small arrow) with low density. Blood cultures and bone marrow biopsy confirmed histoplasmosis. (Reproduced with permission from Radin<sup>236</sup>).

HIV-negative individuals, terminal ileal disease predominates. The radiologic appearance of gastrointestinal histoplasmosis in AIDS includes pseudopolyps or plaques, ulceration, and presence of a localized segment of inflamed thickened bowel.<sup>237</sup> Small bowel barium study or barium enema is useful in demonstrating the lesions in the distal small bowel. Diffuse wall thickening with thickened folds and ulcerations are reported. Partial bowel obstruction or perforation may rarely occur. There may be fistula between adjacent bowel loops and the findings may mimic Crohn's disease or tuberculosis.

Adrenal glands, when involved, are diffusely enlarged with normal shape. There may be regions of necrosis with hypodensity on CT, similar to the spleen and lymph nodes (see Fig. 6-83). Similar to healing splenic granuloma, the adrenal granuloma may calcify. Calcifications may occur later in the course of disease than at clinical presentation. When the calcifications are large, they can be seen on the abdominal radiographs. Adrenal insufficiency was present in five of seven cases reported by Wilson et al.<sup>238</sup> Involvement of the genitourinary tract such as kidneys is rare. *Renal involvement is uncommon in people with normal immunity.* Focal calcifications and hypoechoic areas in the kidneys on sonography due to histoplasmosis were described in an AIDS patient with disseminated disease (Fig. 6-84).<sup>211</sup>

## Paracoccidioidomycosis

Paracoccidioidomycosis is a chronic infectious granulomatous disease caused by *Paracoccidioides brasiliensis*. This is also called South American blastomycosis and is endemic in Central and South America, with the highest incidence in Brazil.<sup>239</sup> *It is the most common pulmonary mycosis in Latin America.* Most of the cases so far reported have been from that region of the world. In a review of 25 patients, lungs (96%), lymph nodes (76%), oropharynx (64%), and adrenals (52%) were the common sites of involvement.<sup>63</sup>

The abdominal involvement leads to extensive and generalized impairment, producing the most diverse clinical



**Figure 6-84** Histoplasmosis involving the kidney in an AIDS patient. Longitudinal renal sonogram shows an echogenic focus (arrow) in the kidney. (Reproduced with permission from Kay<sup>211</sup>).

manifestations including nausea, vomiting, ascites, jaundice, abdominal pain, hepatosplenomegaly, malabsorption syndrome, abdominal mass, intestinal obstruction, peritonitis, and mesenteritis. Biliary involvement can mimic the cholangiocarcinoma. Perforation of the GI tract is also reported. Gastrointestinal tract involvement is seldom recognized clinically.<sup>240</sup> Avritchir and Perroni<sup>241</sup> described three cases with diarrhea in which diagnosis was established only after surgery. On small bowel barium examination, there was thickening of the mucosal folds with focal areas of narrowing and rigidity. Due to the involvement of terminal ileum, appendix and proximal colon, it can simulate tuberculosis, Crohn's disease or lymphoma. Penna<sup>240</sup> described an 8 year old with diffuse ulcerations of the colon and rectal narrowing due to paracoccidioidomycosis.

An autopsy series reported involvement of the adrenal glands in 44–80% of cases. Radiologic findings may vary from adrenal gland enlargement with or without calcification to mass-like appearance. Adrenal enlargement with calcification due to involvement by paracoccidioidomycosis was described.<sup>63</sup> On CT studies the adrenal glands were enlarged with foci of calcifications. Tendrich et al,<sup>242</sup> however, did not find calcifications in any of their 15 cases with adrenal involvement. Recently, Leal et al described a unique pattern of bilateral adrenal masses with central necrosis and peripheral enhancement.<sup>243</sup> Radiologic findings are indistinguishable from other granulomatous diseases, lymphoma, adrenal metastases and hemorrhage. Unless there are other manifestations, diagnosis may only be obtained by percutaneous biopsy.<sup>244</sup>

## Zygomycosis

Zygomycosis is an uncommon fungal infection caused by ubiquitous organisms of the class zygomycetes that have little intrinsic pathogenicity in the normal host. Disseminated zygomycosis is rare but usually fatal. At autopsy, dissemination

to liver, spleen, pancreas, lymph nodes and hollow viscera is reported.<sup>93</sup> GI tract involvement is reported in 7–40% of cases and the stomach is the most frequently involved site, followed by the colon, esophagus and small intestine.<sup>93,245</sup> Zygomycosis produces vascular invasion with thrombosis and ischemic or hemorrhagic infarction of the involved organ. Case reports with imaging findings in abdominal disease have been published.<sup>245</sup> When involved, liver has hypoechoic regions on sonography and hypodense areas on contrast-enhanced CT. There may be a central hyperdensity felt to represent end vessel filled with the fungus causing infarction. Isolated renal involvement is rare.<sup>246</sup> Kidneys are enlarged with hypodense areas on CT when involved.<sup>246</sup> GI tract wall thickening secondary to edema and hemorrhage and rarely pericolic fat proliferation are described.

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# Antifungal therapy

Paul O. Gubbins, Elias J Anaissie

## Historical perspective on the development of antifungal drugs (Fig. 7-1)

Although the existence of fungi dates back a billion years, the history of medical mycology and human mycoses as reviewed by Espinel-Ingroff began in the early 19th century in Italy with the discovery of tinea favosa.<sup>1</sup> In the late 19th century, Gilchrist's report of a human case of blastomycosis in 1894 heralded the beginning of medical mycology in the US.<sup>2</sup> Over the next 60 years landmarks in the field included discoveries and characterization of dimorphic fungi, recognition of fungi as human pathogens capable of causing systemic diseases, the development of laboratory diagnostic tests and classification systems, and initial epidemiologic and ecologic investigations. With these discoveries also came the realization that fungal diseases were prevalent and were becoming more so with advances in medicine. During this period the treatments for these newly discovered diseases were somewhat crude and included mainly surgical therapy. Non-surgical therapies were limited to the use of large doses of potassium iodide, weak acids such as phenol, dyes such as methyl violets or other noxious agents including bromine, potassium permanganate, and oil of turpentine with olive oil.

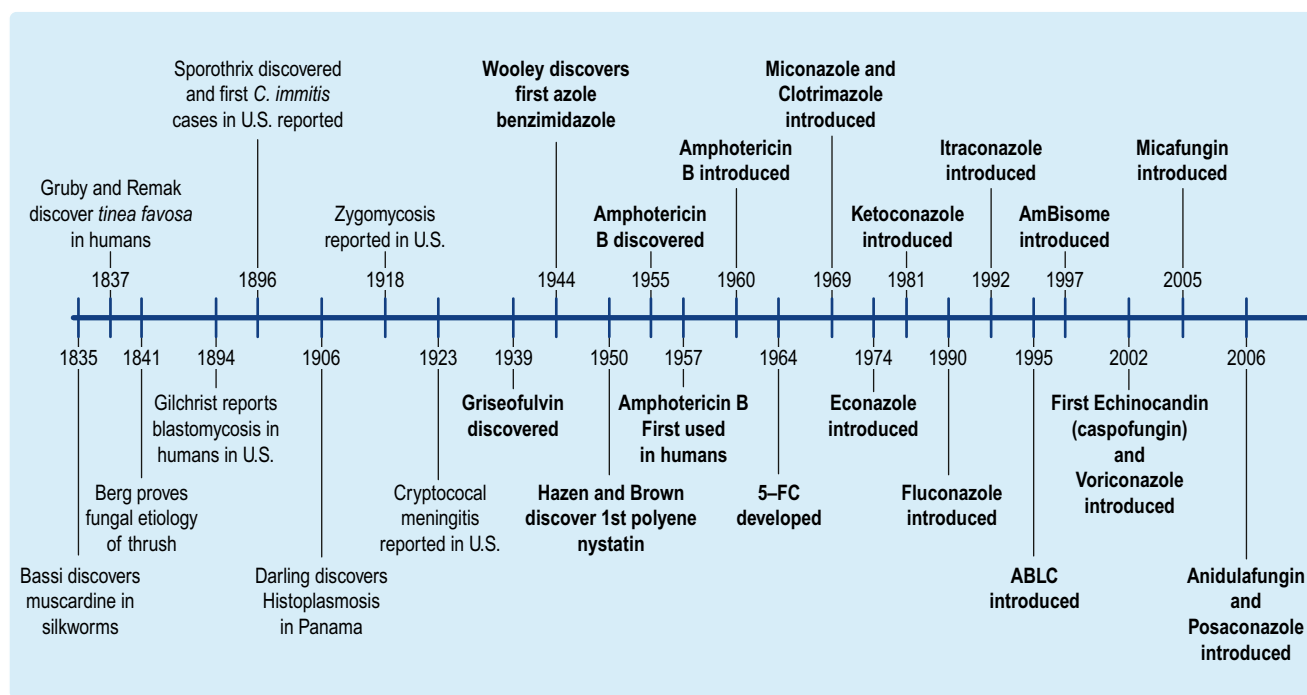
Initial efforts at antifungal therapy were unsuccessful until the demonstration that a saturated solution of potassium iodide (SSKI), taken orally as drops, had some benefit in cutaneous sporotrichosis.<sup>3</sup> Unfortunately, use of SSKI was limited by its very narrow antifungal spectrum activity. As recognition of fungal infections increased, so too did the need for intravenous (IV) or oral antifungal agents.

The first landmarks in the development of active and safe antifungal agents were the discovery of the antifungal activities of griseofulvin by Oxford, in 1939, and the first azole, benzimidazole, by Wooley in 1944.<sup>4-6</sup> Elson's report on the fungistatic properties of propamidine followed in 1945.<sup>7</sup> This was followed by Hazen and Brown's subsequent discovery of the first polyene macrolide antifungal, nystatin, in 1950, which had important implications for the modern era of antifungal therapy.<sup>8,9</sup> In 1951, propamidine and a related compound

stilbamidine were used in a few human cases of blastomycosis with limited success, but with notable toxicity.<sup>10</sup> Two years later a less toxic stilbamidine derivative, 2-hydroxystilbamidine, was used in three additional human cases of blastomycosis, with limited success.<sup>11</sup> The discovery of amphotericin B (AmB) in 1955 and subsequent reports of its use to treat several human cases of blastomycosis in 1957 illustrate the speed with which the search for effective and safe antifungal agents was progressing.<sup>12,13</sup> The introduction of oral griseofulvin and topical chlormidazole in 1958 and the subsequent introduction of IV AmB in 1960 heralded the beginning of the modern era of antifungal therapy.<sup>4</sup>

Unfortunately, after the introduction of AmB the discovery of new safer and effective agents proved somewhat elusive and advances in the search for new antifungal agents slowed throughout the next three decades. Developed in 1964, the oral agent 5-fluorocytosine (flucytosine – 5FC) was initially effective in the treatment of infections caused by *Candida albicans* and *Cryptococcus neoformans*; however, the development of 5FC resistance soon limited its use as monotherapy. Nonetheless, 5FC is still used in combination with AmB in the treatment of cryptococcal meningitis.<sup>1</sup> Miconazole and clotrimazole were both introduced as topical agents in 1969, and represented the only two additions to the antifungal armamentarium in the 1960s.

The 1970s saw the development of the imidazole antifungals, which possessed broad-spectrum activity against dermatophytes, *Candida*, and other fungi. The topical agent econazole was developed in 1974 and is still available today. During this decade attempts to develop systemic formulations of clotrimazole and miconazole were made, with limited success.<sup>14-17</sup> The introduction of ketoconazole in 1981 represented the nadir in the search for new safer and effective agents. For nearly a decade it was the only oral agent available for the treatment of systemic fungal infections. However, the 1970s and 1980s were not devoid of discoveries, including initial research on the allylamines (naftidine) and polyene lipid formulations, and the discovery of fluconazole and the echinocandins or their precursors. However, it would be approximately a decade before the significance of these discoveries was realized.<sup>4</sup>



**Figure 7-1** History of antifungal development.

The 1990s were perhaps the most prolific period in antifungal development. When the decade began, clinicians essentially had one agent for IV use and one agent for oral use to choose from when treating systemic fungal infections. However, by the end of the decade, antifungal agents were becoming distinguishable in terms of activity, toxicity, and drug interaction potential to allow clinicians to differentiate between agents and tailor therapy to suit specific patient needs. In 1990, the introduction of fluconazole transformed antifungal development. Fluconazole, the first broad-spectrum triazole, addressed the principal shortcomings of the imidazoles: poor solubility and lack of an IV formulation. In 1992, itraconazole was introduced and expanded the spectrum of activity of the triazole class beyond *Candida* spp. to include a variety of filamentous fungi.<sup>18</sup> Eventually these triazoles supplanted ketoconazole as the treatment of choice for many systemic mycoses. In the mid-1990s the solubilizing excipient hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ CD) enhanced itraconazole bioavailability by enabling the development of the oral and IV solution formulations. During this time safer lipid-based formulations of the polyenes AmB and nystatin were also introduced.

The expansion in antifungal development continued into the new millennium with the advent of the first new class of antifungal agents in nearly 40 years, the echinocandins, with the introduction of caspofungin in 2001. Since 2001, the echinocandin class has continued to expand with the introduction of micafungin and anidulafungin, and the triazole class has expanded with the addition of voriconazole and posaconazole, both with increased activity against fluconazole-resistant *Candida* spp. and filamentous moulds.

Table 7-1 shows the antifungal agents approved for the treatment of systemic mycoses in the US.

## Pharmacology of antifungal agents

### Antifungal targets

Unlike the development of antibacterial agents, to date relatively few drug targets in fungi have been exploited in the development of currently available antifungal agents. Antibacterial agents have taken advantage of multiple targets available in bacteria that are not present in mammalian cells. Fungi have similarities to mammalian cells that have made the search for antifungal drug targets difficult. To date, three targets – plasma membrane sterols, nucleic acid synthesis and cell wall constituents (chitin,  $\beta$ 1,3-glucan, and mannoproteins) – have been exploited with varying degrees of success (Fig. 7-2).

Most of the current antifungal agents available for systemic use rely on the direct (the polyenes) or indirect (the azoles) interaction with the plasma membrane sterol ergosterol. The cell wall-acting echinocandin class of antifungal agents was the first major class of systemically acting antifungals to exploit a unique target,  $\beta$ 1,3 glucan synthase. This rapidly growing area of research will continue to be important as the need for potent, less toxic antifungal agents continues to increase.

### Plasma membrane sterols: ergosterol and ergosterol synthesis

To provide structural integrity, the fungal cell membrane is composed of sterols lacking C-4 methyl groups such as ergosterol.<sup>19</sup> Ergosterol, a key component of the fungal cell membrane, is critical to the integrity of the membrane and functions by regulating membrane fluidity and asymmetry.<sup>19</sup> This sterol



**Table 7-1** Drugs approved for the treatment of systemic mycoses in the United States

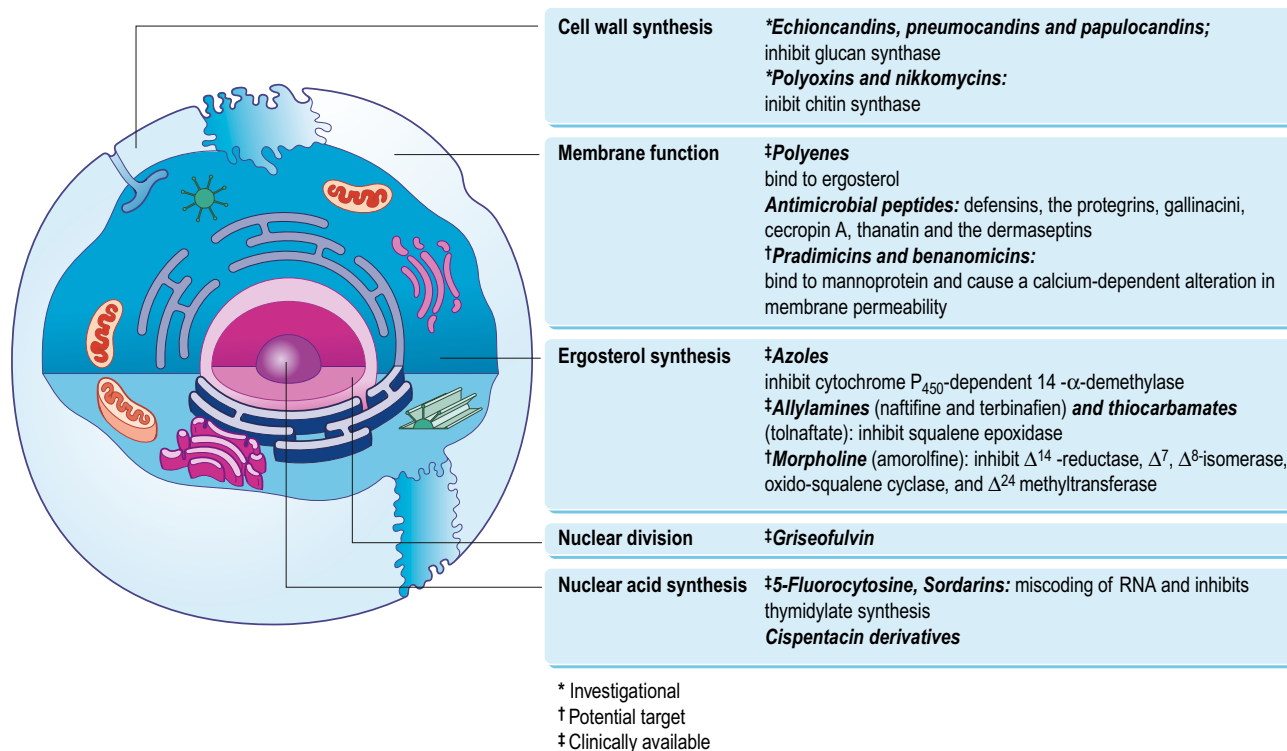
<b>Class</b>	<b>Mechanism of action</b>	<b>Generic name</b>	<b>Brand name</b>	<b>Available formulation</b>
Polyenes	Destabilizes the fungal cell membrane. Binds to the sterol ergosterol incorporated in the fungal cell membrane, which creates pores in the membrane and leads to depolarization of the membrane with subsequent cell leakage. In mammalian cells polyenes bind cholesterol	Amphotericin B deoxycholate	Fungizone	IV, oral solution
		Amphotericin B lipid complex	Abelcet (ABLC)	IV
		Amphotericin B colloidal dispersion	Amphotec (ABCD)	IV
		Amphotericin B liposomal	AmBisome (LAmB)	IV
Pyrimidine	Transported intracellularly via cytosine permease. Converted to fluorouracil via cytosine deaminase, and subsequently converted to 5-fluorouridine triphosphate, which is incorporated into fungal RNA and interferes with protein synthesis. 5FC intermediate also inhibits thymidylate synthetase, and interferes with DNA synthesis	Flucytosine (5FC)	Ancoban	Oral tablet
Azoles	Interferes with sterol synthesis via inhibition of CYP-dependent C-14 $\alpha$ demethylase, a fungal CYP enzyme important in converting lanosterol to ergosterol	Ketoconazole	Nizoral	Oral tablet
		Fluconazole	Diflucan	IV, oral suspension, oral tablet
		Itraconazole	Sporanox	Oral capsule, oral solution
		Voriconazole Posaconazole	Vfend Noxafil	IV, oral tablet Oral suspension
Echinocandins	Inhibition of $\beta$ 1,3-glucan sythesis via inhibition of $\beta$ 1,3-glucan synthase. Fungal cell wall is mosly polysaccharides, and glucans are the most abundant polymers in fungal cell walls. Glucan synthase catalyzes polymerization of these polysaccharides. Inhibition of this unique enzyme ultimately leads to increased cell wall permeability and lysis of the cell	Caspofungin Micafungin Anidulafungin	Cancidas Mycamine Eraxis	IV IV IV

Key: IV, intravenous; 5FC, 5-fluorocytosine or flucytosine; CYP, cytochrome P450.

is not present in mammalian cells and thus it is an ideal target for antifungal activity. Most currently available antifungal drugs interact with or inhibit the synthesis of ergosterol. The polyene antifungals bind directly to membrane sterols (especially ergosterol) and form ionic transmembrane channels. These channels cause an increase in membrane permeability that leads to leakage of intracellular contents, including potassium, and eventual cell death.<sup>20,21</sup>

Indirectly, ergosterol is targeted by a variety of antifungal agents that act at one or more steps in the biosynthesis

of ergosterol. One such target in the ergosterol biosynthesis pathway is cytochrome P450 (CYP)-dependent 14 $\alpha$ -demethylase, which catalyzes the demethylation of ergosterol precursors. Certain azoles (i.e., ketoconazole, voriconazole) may interact with secondary targets in the ergosterol biosynthesis pathway; 14 $\alpha$ -demethylase is the primary target for this class of compounds.<sup>19,20,22,23</sup> Inhibition of 14 $\alpha$ -demethylase ultimately causes the depletion of ergosterol and the accumulation of sterol precursors, including 14 $\alpha$ -methylated sterols (lanosterol, 14-dimethyl lanosterol, and



**Figure 7-2** Sites of action of antifungals.

24-methylenedihydrolanosterol) (Fig. 7-3). This inhibition results in the formation of a plasma membrane with diminished structural integrity and altered function, which manifests as fungistatic activity.<sup>19</sup>

Squalene epoxidase is another target in the ergosterol biosynthesis pathway, inhibition of which can lead to either fungistatic or fungicidal effects.<sup>24-27</sup> Allylamines (terbinafine) and thiocarbamates (tolnaftate) act here and have minimal cross-reactivity with the mammalian enzyme involved in cholesterol synthesis.<sup>24</sup> The morpholine amorolfine, a topical antifungal agent for the treatment of onychomycosis, which is not available in the US, acts via inhibition of  $\Delta^{14}$ -reductase and  $\Delta^7$ ,  $\Delta^8$ -isomerase, which are also enzymes in ergosterol synthesis.<sup>28,29</sup> Undoubtedly, many more clinically useful antifungal agents will be discovered as the search for newer, more potent ergosterol synthesis inhibitors continues.

## Nucleic acid synthesis

Only one of the currently available agents, flucytosine (5-fluorocytosine, 5FC), targets nucleic acid synthesis. 5FC is transported into the fungal cell by cytosine permease. Intracellularly, it is converted to 5-fluorouracil via cytosine deaminase, and subsequently converted to 5-fluorouridine triphosphate, which is incorporated into fungal RNA to cause early chain termination.<sup>30</sup> The triphosphate is also ultimately converted to 5-fluorodeoxyuridine monophosphate, which also inhibits thymidylate synthetase, thereby interrupting DNA synthesis as well.<sup>30</sup> No further agents directed at this target have been marketed.

## Fungal cell wall: glucans

The fungal cell wall is uniquely composed of mannoproteins, chitins, and  $\alpha$ - and  $\beta$ - linked glucans and serves many functions, including providing cell rigidity and shape, metabolism, ion exchange, and interactions with host defense mechanisms. The composition of the cell wall varies between species of fungi but a major component of many fungal cell walls is  $\beta$ 1,3-glucan. The echinocandins, semi-synthetic lipopeptides derived from fungi, non-competitively inhibit  $\beta$ 1,3-D-glucan synthase, blocking the synthesis of  $\beta$ 1,3-glucan. This lessens cellular structural integrity and morphology and ultimately results in osmotic lysis of the cell.<sup>31</sup>

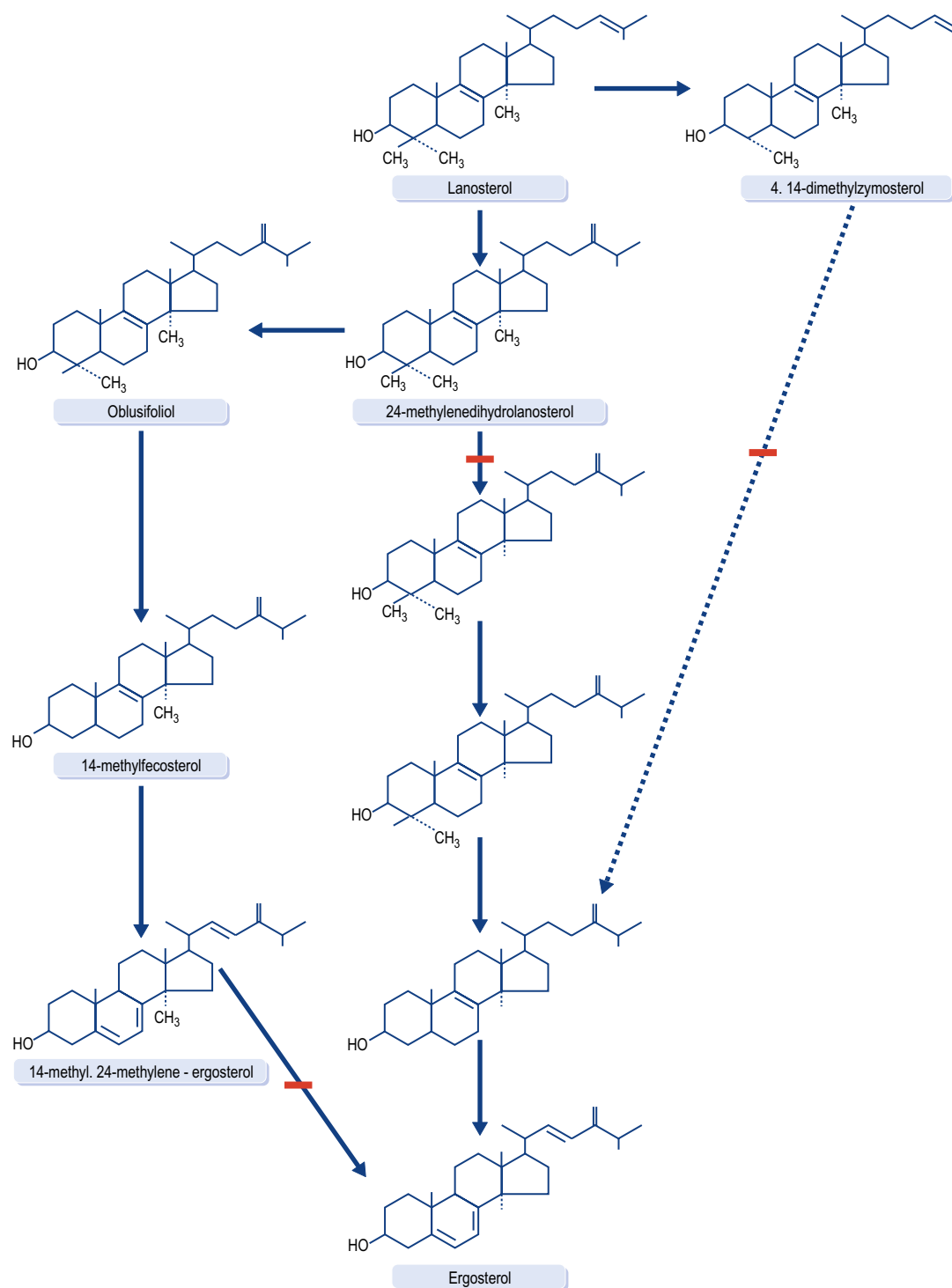
## Other targets under development

### Nucleic acid synthesis

Several antifungals under development target fungal DNA or RNA.<sup>32</sup> Yatakemycin is a natural product isolated from *Streptomyces* spp. that acts via sequence-specific DNA alkylation.<sup>33</sup> Icofungipen is an orally administered, synthetic derivative of the naturally occurring  $\beta$ -amino acid cispetacin, which was originally isolated from *Bacillus cereus*.<sup>34</sup> This compound blocks isoleucyl-tRNA synthetase, resulting in the inhibition of protein synthesis and growth of fungal cells.<sup>34</sup>

### Fungal cell wall

In addition to glucans, the fungal cell wall also contains chitins, which are present in smaller quantities and function to provide a framework for the cell wall.<sup>32,35,36</sup> Polyoxins and



**Figure 7-3** Pathway for sterol synthesis of ergosterol and blocks by azoles.

nikkomycins are chitin synthase inhibitors which have been pursued for development. These compounds are substrate analogs of UDP-*N*-acetylglucosamine, the foundation of chitin biosynthesis.<sup>32,37</sup> Mannoproteins are another major component of the outer cell wall and may help determine its morphology.<sup>35</sup> Pradimicins and benanomycins are mannoprotein-binding

antifungal agents that have been studied.<sup>37</sup> Their exact mechanism of action has not been defined.<sup>37</sup> It remains to be seen whether these and other targets that have or may be discovered in the fungal cell wall show promise for the development of future antifungal agents; nonetheless, some are undergoing clinical trials.



### Other Novel Targets Under Development

Other potential targets, including protein synthesis pathways, have been identified in fungi, although few antifungal compounds have been synthesized to take advantage of them. Figure 7-2 summarizes the targets used by marketed or investigational antifungal agents. One target that has been studied for many years is elongation factor 2 (EF2). The sordarins are the most studied class of compounds directed at this target. These derivatives are specific inhibitors of fungal translation factor EF2, which catalyzes the translocation of tRNA and mRNA after peptide bond formation.<sup>32,38</sup> The *N*-myristylation of fungal proteins is also a potential target to exploit. *N*-myristoyl proteins, also known as ADP-ribosylation factors, are essential to fungal growth and potent inhibitors are fungicidal.<sup>32,39,40</sup> Protein *N*-myristoyl transferase is critical for in vitro viability of *C. albicans* and *C. neoformans*.<sup>32</sup> The naturally occurring cationic peptides and their synthetic derivatives are potentially promising agents. These peptides include the defensins, the protegrins, gallinacin, cecropin A, thanatin and the dermaseptins. There are other novel targets including intracellular organelles, specific enzymes involved in the sphingolipid biosynthesis pathway, to name a few. However, whether investigations into these targets will yield clinically useful antifungal agents remains to be seen.

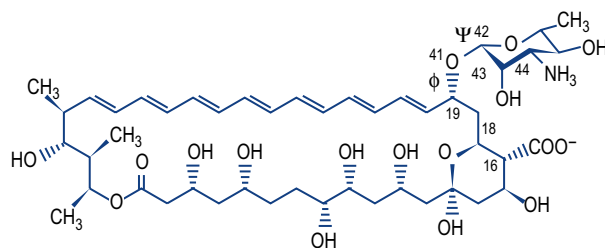
## Currently available systemic antifungal drugs by class

### Polyenes

#### Amphotericin B formulations

**Mechanism of action** The polyenes (AmB, nystatin) are large (26–28 carbon molecules) macrolide structures, with many hydroxyl groups, which confer the amphipathic nature of the compounds (Fig. 7-4). Of the initially discovered polyenes, only AmB is sufficiently benign to permit IV administration.<sup>32,41</sup> Amphotericin B is amphiphilic and acts by binding through both hydrophilic hydrogen bonds and hydrophobic, non-specific van de Waals forces to ergosterol in fungal cell membranes.<sup>42–44</sup> Amphotericin B has a greater affinity to bind ergosterol and ergosterol-containing membranes than cholesterol or cholesterol-containing membranes.<sup>42,45,46</sup> Conformationally, compared to cholesterol, the chemical structure of ergosterol is more favorable for interactions governed by van der Waals forces.<sup>42</sup> The binding occurs within minutes of exposure and is followed by increasing leakage of intracellular ions out of fungal cells (i.e., potassium) and extracellular ions into cells, which leads to depolarization of the membrane and increased permeability to protons and monovalent cations.<sup>43,47,48</sup> This osmotic disruption may not be the main mechanism of lethality to fungal cells, because polyenes also interfere with membrane-associated oxidative enzyme function, and this secondarily is thought to be lethal.<sup>43,49</sup>

Although rapid lethal action is clearly shown in vitro against a number of fungal pathogens, neither polyenes nor any other antifungal drugs are lethal in vivo.<sup>50–52</sup> It is not clear whether this is due to a protected intracellular environment of some pathogens or to limited access to fungal targets.



**Figure 7-4** Structure of amphotericin B.

In addition to direct antifungal activity, AmB stimulates release of cytokines such as tumor necrosis factor and interleukin-1 from mammalian phagocytic cells and also stimulates release of macrophage superoxide ion, all of which may augment antifungal activity.<sup>53–55</sup>

**Spectrum of activity** The antifungal spectrum of AmB is extremely broad, it being easier to list the few exceptions than the targeted species. However, growing evidence suggests less than optimal activity against a number of *Candida* species.<sup>56</sup> *Candida lusitanae* has long been recognized to develop resistance to AmB upon exposure to the drug.<sup>43</sup> Moreover, increased minimum inhibitory concentrations (MIC) have been described for less common species including *C. guilliermondii* and *C. rugosa*, and rare mutants of *Candida* species, including *C. albicans*, *C. tropicalis*, and others.<sup>57</sup> Initial reports demonstrated that *C. guilliermondii* was capable of developing resistance to AmB; however, only 2% of bloodstream isolates from a large global surveillance were resistant to the drug.<sup>57,58</sup> The diminishing susceptibility to AmB among more common *Candida* spp., including *C. glabrata* and *C. krusei*, is a growing concern.<sup>59</sup> Both *C. glabrata* and *C. krusei* exhibit decreased susceptibility to AmB compared with *C. albicans*.<sup>60–62</sup> Moreover, AmB also exhibits delayed killing against these species when compared to its activity against *C. albicans*.<sup>63</sup>

Although AmB is active against most moulds, there is interspecies variability with respect to amphotericin MICs. Among *Aspergillus* spp., *A. terreus*, *A. flavus* and *A. nidulans* typically are less susceptible to AmB than other species.<sup>64–67</sup> Moulds other than *Aspergillus* spp., including certain zygomycetes, *Fusarium* spp., *Pseudallescheria* spp., *Scedosporium* spp., *Exophiala* spp., *Alternaria* spp., *Cladosporium* spp. and *Trichosporon asahii* (formerly *T. beigelii*), also exhibit high (2–32 µg/ml) AmB MICs.<sup>64,68</sup>

Table 7-2 describes the antifungal spectrum of AmB according to clinical response.

**Pharmacokinetics** In humans, AmB primarily distributes to the liver and, to a lesser extent, a variety of other tissues including the spleen, kidneys, and heart.<sup>41</sup> Amphotericin B deoxycholate (D-AmB) is highly protein bound (>95%), primarily to albumin and  $\alpha_1$ -acid glycoprotein.<sup>69</sup> The percentage of bound drug increases as the D-AmB concentration increases. This unique binding may be due to the low solubility of unbound D-AmB in human plasma (<1 µg/ml), relative to the large binding capacity of plasma proteins.<sup>69</sup> Amphotericin B deoxycholate has a very large apparent volume of distribution (2–4 l/kg), suggesting that it distributes to tissues.<sup>69,70</sup>

Historically, D-AmB pharmacokinetics have been poorly understood, but over the years our understanding of how the human body handles D-AmB has greatly improved. Over

**Table 7-2** Antifungal spectrum of Amphotericin B by clinical response

Usually Effective (>60%)	Variably Effective to Resistant
<i>Candida albicans</i>	<i>Candida lusitanae</i>
<i>Candida krusei</i>	<i>Candida rugosa</i>
<i>Candida tropicalis</i>	<i>Fusarium</i> spp.
<i>Candida parapsilosis</i>	<i>Pseudallescheria boydii</i>
<i>Cryptococcus neoformans</i> var. <i>neoformans</i> /var. <i>gattii</i>	<i>Scedosporium prolificans</i>
<i>Histoplasma capsulatum</i> var. <i>capsulatum</i> /var. <i>duboisii</i>	Various
<i>Paracoccidioides brasiliensis</i>	Phaeohyphomycetes
<i>Blastomyces dermatitidis</i>	<i>Aspergillus</i> spp.
<i>Penicillium marneffei</i>	<i>Coccidioides immitis</i>
<i>Sporothrix schenckii</i>	

Note that clinical response does not always correlate with in vitro response. For example, *Aspergillus* species and *Coccidioides immitis* are usually susceptible in vitro to amphotericin B, but clinical responses vary widely because of other variables such as immune response.

90% of the administered dose is accounted for 1 week after the administration of 0.6 mg/kg D-AmB to healthy volunteers, with most of the drug being excreted unchanged in the feces and urine.<sup>70</sup> Approximately two-thirds of D-AmB is recovered in the urine (20.6%) and feces (42.5%).<sup>70</sup> Urinary and fecal clearances of unchanged drug account for 75% of D-AmB total clearance.<sup>70</sup> Amphotericin B deoxycholate is apparently cleared from its distribution sites very slowly, as reflected by its terminal half-life of approximately 127 hours.<sup>70</sup> The formulation of amphotericin B in a lipid vehicle significantly alters its distribution and elimination.<sup>70</sup>

The lipid amphotericin B formulations differ in composition and physicochemical properties. These differences produce subtle differences in amphotericin B pharmacokinetics among the formulations (Table 7-3). Following IV administration, lipid amphotericin B formulations are cleared from the circulation by macrophages or monocytes, Kupffer cells lining the hepatic sinusoids, reticular cells of the lymphatic tissue and bone marrow, spleen, and lung.<sup>71</sup> Physicochemical properties (size, surface charge, and composition and stability) of these compounds also determine how quickly they are cleared from the circulation. In general, the smaller the liposome or lipid complex, the longer it circulates. In addition, positively charged or neutral liposomes or lipid complexes circulate longer than those of similar size that are negatively charged.<sup>71,72</sup> Lipid amphotericin B formulations can also be broken down in the serum or degraded by phospholipases. When amphotericin B is incorporated into liposomes or associated with lipid complexes, the lipids only influence its distribution in the body.

Amphotericin B lipid complex (ABLC) is 1.6–11  $\mu$ m in size. Because of its size and surface charges, it is cleared rapidly from the circulation and distributes extensively to tissue. Consequently, compared to equivalent doses of the deoxycholate formulation, ABLC produces lower maximum serum concentrations and drug exposure while at steady state, and its volume of distribution and total clearance are higher.<sup>73</sup>

Liposomal amphotericin B (L-AmB) consists of spherical liposomes, 45–80 nm in size, with an aqueous core.<sup>74</sup> Due to its small particle size distribution compared to other amphotericin B formulations, L-AmB is more slowly cleared from the bloodstream, has a longer circulation half-life, and achieves higher maximum plasma concentration and systemic drug exposure, but has a smaller volume of distribution.<sup>75</sup> In addition, less than 10% of a dose is recovered in the urine and feces.<sup>70</sup>

Amphotericin B colloidal dispersion (ABCD) is a stable complex of amphotericin B and cholesteryl sulfate in a 1:1 molar ratio. This lipid formulation exists as disk-like structures averaging 75–170 nm.<sup>72</sup> The pharmacokinetics of this formulation are similar to those of the deoxycholate and lipid complex formulations.

Figure 7-5 shows the structure of the lipid formulations of amphotericin B.

Whether the subtle pharmacokinetic differences described above impact on clinical efficacy is still debatable. In addition to their physicochemical and pharmacokinetic properties, the in vivo activity of these formulations likely also depends on the amphotericin B concentration at the site of infection. However, there are very few published data describing amphotericin B disposition in human tissue following administration of a lipid amphotericin B formulation. Data from autopsy material of patients who had been treated with L-AmB or ABCD for suspected or proven invasive fungal infection suggest that individual differences in lipid amphotericin B formulation may influence amphotericin B penetration into the lung.<sup>76</sup> Amphotericin B lung concentrations in patients treated with ABCD significantly exceeded those of the liposomal amphotericin B-treated patients.<sup>76</sup> Both formulations produced high concentrations in the liver and spleen, and low concentrations in the myocardium and kidney. However, amphotericin B kidney concentrations in patients treated with ABCD significantly exceeded those of the liposomal amphotericin B-treated patients.<sup>76</sup>

Amphotericin B tissue concentrations following administration of lipid amphotericin B formulations have been compared to those following administration of the deoxycholate formulation in animal models. In these models, compared to the deoxycholate formulation, all lipid amphotericin B formulations produce higher amphotericin B concentrations in the liver, spleen and, in the case of ABLC, in the lungs.<sup>77</sup>

The central nervous system (CNS) disposition and antifungal efficacy of all the lipid amphotericin B formulations have also been studied in an animal model. Data suggest that amphotericin B delivery to the CNS following administration of a lipid amphotericin B formulation is a function of a concentration gradient between the plasma and CNS. The lipid amphotericin B formulations that do not achieve high and sustained concentrations of free compound in the plasma may not be successful in eradicating species such as *C. albicans* from brain tissue.<sup>78</sup> Of the three lipid amphotericin B formulations, L-AmB achieves high and sustained concentrations of free compound in the plasma and consequently, it was the most successful in eradicating *C. albicans* from brain tissue.<sup>78</sup>

The lipid amphotericin B formulations distribute similarly into bone marrow, liver, and fat tissue of uninfected animals. All of the lipid amphotericin B formulations achieve markedly higher concentrations in the bone marrow and liver than the D-AmB formulation, with ABCD demonstrating the greatest degree of distribution into these sites.<sup>79</sup> Amphotericin B lipid

**Table 7-3** Comparison of the pharmacokinetics between the amphotericin B formulations

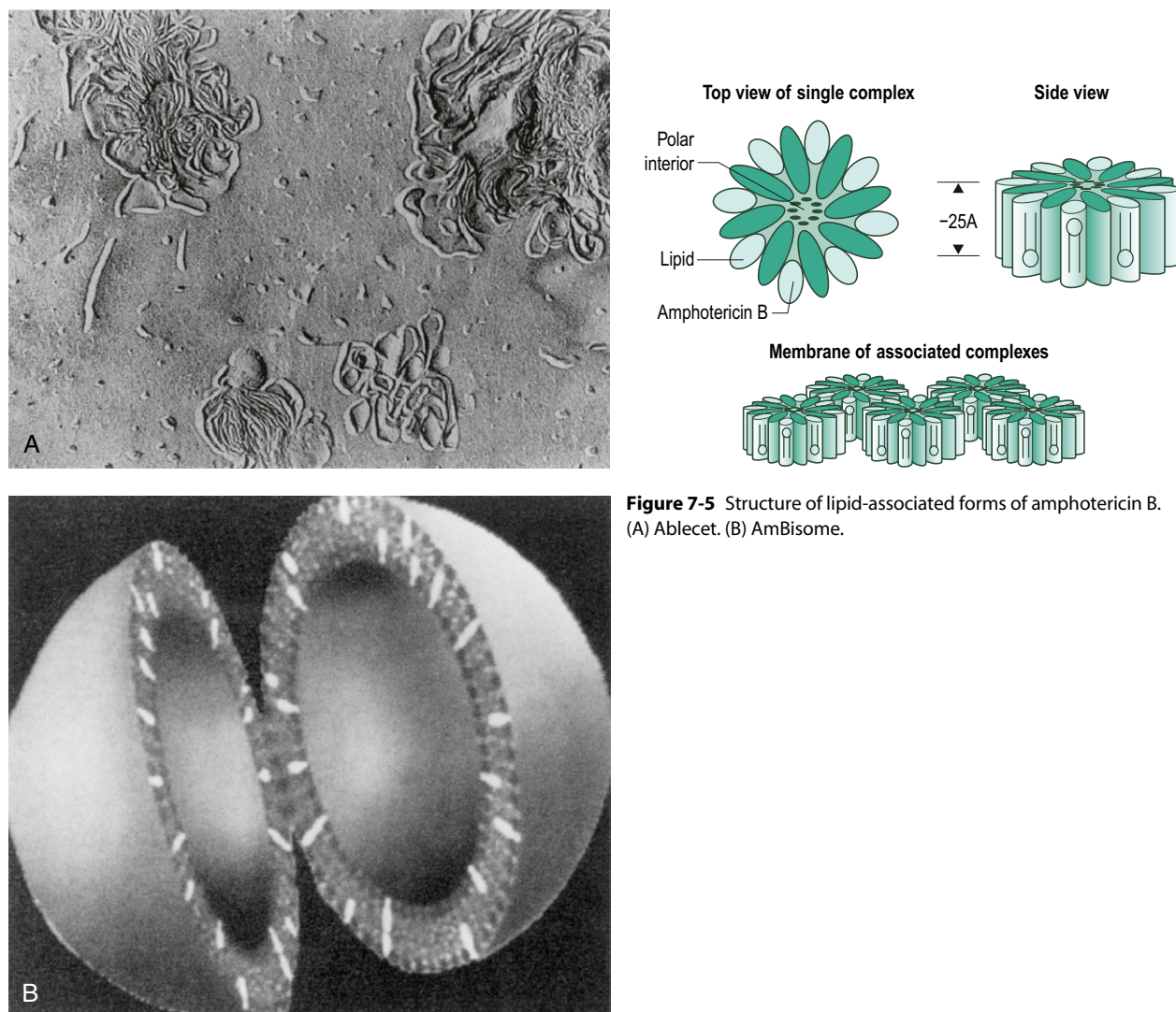
Formulation	Relative to amphotericin B deoxycholate										
	Molecular size (µm)	Mean human pharmacokinetic parameters			Tissue distribution in rabbit models simulating human pharmacokinetics						
		C <sub>max</sub>	Vd	Cl	AUC	Brain	Bone marrow	Liver	Fat <sup>a</sup>	Lung	Comments
Amphotericin B lipid complex	1.6-11	↓	↑	↑	↓	↔	↑	↑	↔	↑ <sup>b</sup>	Brain ABLC concentrations greater in infected vs uninfected animals; ABLC concentrations highest in rabbit lung tissue and pulmonary alveolar macrophages
Amphotericin B colloidal dispersion	0.075-0.17	↓	↑	↔	↓	↔	↑ <sup>b</sup>	↑ <sup>b</sup>	↔	↔	Brain ABCD concentrations greater in infected vs uninfected animals; Human autopsy data – ABCD demonstrated greater distribution into lung, spleen, kidney than liposomal amphotericin B
Liposomal amphotericin B	0.045-0.08	↑	↓	↓	↑	↑ <sup>b</sup>	↑	↑	↔ <sup>b</sup>	↔	Brain liposomal amphotericin B concentrations similar in infected vs uninfected animals; liposomal amphotericin B concentrations highest in rabbit epithelial lung fluid. Human autopsy data – liposomal amphotericin B demonstrated greater distribution to liver than ABCD

Key: AmB, amphotericin B; µm, microns; C<sub>max</sub>, maximum concentration; Vd, volume of distribution; Cl, clearance; AUC, area under the serum concentration time curve; Scr, serum creatinine; ABLC, amphotericin B lipid complex; ABCD, amphotericin B colloidal dispersion; LAmB, liposomal amphotericin B; ↑, increased; ↓, decreased; ↔, similar;

<sup>a</sup>all preparations demonstrate poor penetration;

<sup>b</sup>greatest degree of penetration among formulations.





**Figure 7-5** Structure of lipid-associated forms of amphotericin B. (A) Ablecet. (B) AmBisome.

complex and L-AmB achieved concentrations 2–4 times and 2–5 times those of the D-AmB formulation in the liver and bone marrow, respectively.<sup>79</sup> In contrast to liver and bone marrow, all lipid amphotericin B formulations accumulate poorly within fat tissue.<sup>79</sup>

**Toxicity** The toxicities of D-AmB are dose and infusion related. Immediately after administration, amphotericin B dissociates from the micelles and largely binds to low-density lipoprotein in the plasma.<sup>80–82</sup> Then it binds preferentially to fungal cell membrane ergosterol but also binds less avidly to mammalian cell membrane cholesterol. High concentrations of amphotericin B can damage erythrocytes and other mammalian cells and cause osmotic leakage of hemoglobin and intracellular ions.<sup>83</sup>

Although hyperkalemia may occur with rapid infusions, administration of D-AmB normally leads to renal tubular potassium wasting and distal renal tubular acidosis, which can be treated with potassium and bicarbonate.<sup>41</sup> Other dose-related toxicities include nephrotoxicity, azotemia, electrolyte imbalance, cardiac arrhythmias, and anemia.<sup>41</sup> Amphotericin B deoxycholate-induced nephrotoxicity is the primary “dose-related” toxicity, and its incidence varies from 15% to 80%,

depending on the population of patients.<sup>41</sup> With repeated dosing, amphotericin B also causes glomerular vasospasm and ischemia, resulting in decreases of glomerular filtration rate. Risk factors for D-AmB induced nephrotoxicity include average daily dose, concomitant nephrotoxin (particularly cyclosporine) use, and elevated baseline serum creatinine.<sup>84,85</sup>

In some patient populations, glomerulotubular flow may be augmented by acute infusion of isotonic saline before amphotericin B and may somewhat preserve renal function.<sup>86</sup> However, the usefulness of saline hydration may be offset by fluid restriction employed to manage the fluid status of critically ill patients. As renal failure progresses, synthesis of erythropoietin diminishes, which leads to anemia.<sup>87</sup> Most cases of D-AmB induced nephrotoxicity are mild to moderate in nature and reversible in patients at low risk for this complication.<sup>88</sup> Severe nephrotoxicity is uncommon but it too is often reversible.<sup>88</sup> Although D-AmB is nephrotoxic, renal dysfunction does not significantly impact its kinetics. Therefore, anephric patients may be treated with dosages similar to those given to patients with normal renal function. Initial decreases in renal function caused by amphotericin B may be improved by occasional interruption of therapy, switching to the lipid amphotericin B

formulations that cause less nephrotoxicity or to a class of compounds such as the echinocandins or azoles that do not cause nephrotoxicity.

Amphotericin B deoxycholate causes “infusion-related” reactions, including hypotension, fever, rigors, and chills, in approximately 70% of patients.<sup>84</sup> These “infusion-related” reactions occur early in therapy and often subside with time. Pretreatment regimens consisting of diphenhydramine, acetaminophen, meperidine, and hydrocortisone are used to prevent “infusion-related” reactions (Table 7-4). The “infusion-related” reactions are bothersome and may cause early discontinuation of D-AmB therapy or may interfere with the use of other agents.

In addition to nephrotoxicity and infusion toxicities, amphotericin B produces local thrombophlebitis, nausea, and vomiting.<sup>72,89</sup> These toxicities may also be minimized to varying degrees by pretreatment regimens. Morphine and meperidine have also been used to relieve these symptoms.<sup>84</sup> Renal failure and anemia are the main toxicities that are significantly reduced by the use of lipid amphotericin B formulations.<sup>90-92</sup>

Commercially available lipid amphotericin B formulations represent a significant advance in antifungal therapy and their safety and efficacy have been extensively reviewed.<sup>90,91</sup> In empiric antifungal therapy settings, no significant difference in efficacy has been detected between lipid amphotericin B formulations and D-AmB.<sup>92</sup> However, all the lipid amphotericin B formulations lower the risk of D-AmB induced nephrotoxicity. Although ABCD is less nephrotoxic than D-AmB, a large double-blind randomized study demonstrated that infusion-related adverse events were significantly more common with ABCD than with D-AmB and 16 patients reported hypoxic events that were likely attributable to their study drug.<sup>93</sup> These events occurred primarily in patients who received ABCD, and the vast majority of these episodes were associated with required oxygen supplementation. In several patients, the hypoxic events resulted in the early discontinuation of ABCD therapy.<sup>93</sup> Because of these data, ABCD is not widely used. In contrast, ABLC and particularly L-AmB have reduced rates of infusion toxicities and nephrotoxicity (defined as doubling serum creatinine) compared with D-AmB.<sup>92</sup> In a double-blind study to compare safety of ABLC and L-AmB, neutropenic patients with persistent fever were randomized to receive ABLC at 5 mg/kg/d (n = 78), L-AmB at 3 mg/kg/d (n = 85), or at 5 mg/kg/d (n = 81). L-AmB (3 mg/kg/d and 5 mg/kg/d) had significantly lower rates of fever and chills/rigors on day 1, nephrotoxicity, and toxicity-related discontinuations of therapy. Therapeutic success was similar in all 3 groups.<sup>92b</sup> **This reduced toxicity allows increased doses of antifungal therapy to be utilized.** With their superior safety profiles, ABLC and L-AmB are considered suitable alternatives to D-AmB.<sup>94</sup>

Amphotericin B deoxycholate is administered IV as a micelle mixture in 5% glucose. Admixture or infusion with saline must be avoided, because it causes precipitation of the micelles. Pharmacy reconstitution instructions are different for each preparation and should be carefully followed. Although the drug may be administered as a rapid infusion (45–60 minutes), there really is no medical reason for this and conventionally all formulations of amphotericin B are infused over 2–4 hours. The infusions should be much slower in patients with renal insufficiency.<sup>41</sup>

## Fluorinated pyrimidine analog

### Flucytosine

**Mechanism of action** Flucytosine is the lone member of the group of fluorinated pyrimidine analog antifungal compounds. To exert its effect, flucytosine is taken up in susceptible fungi by the transport enzyme cytosine permease. This uptake can be competitively antagonized by adenine, hypoxanthine and cytosine, which all share this transport system.<sup>95</sup> Once inside the fungal cell, flucytosine rapidly undergoes intracellular conversion to 5-fluorouracil via cytosine deaminase.<sup>96</sup> Fungi lacking cytosine deaminase are intrinsically resistant to flucytosine.<sup>96</sup> After intracellular conversion to 5-fluorouracil the antifungal effect is exerted via one of two mechanisms. These mechanisms are independent of each other but whether both are responsible for flucytosine activity is unknown.<sup>97</sup> First, through a series of phosphorylation reactions, 5-fluorouracil is ultimately converted to its triphosphate form, 5-fluorouridine triphosphate.<sup>97</sup> The triphosphate form is incorporated into fungal RNA in place of uridylic acid, which alters the amino acylation of tRNA and ultimately inhibits protein synthesis.<sup>97</sup> A secondary mechanism ultimately leads to inhibition of DNA synthesis and involves the metabolism of 5-fluorouracil into 5-fluorodeoxyuridine monophosphate by uridine monophosphate pyrophosphorylase. 5-fluorodeoxyuridine monophosphate is a potent inhibitor of thymidylate synthetase, which is critical to DNA biosynthesis.<sup>98</sup> The importance of this secondary mechanism of activity is unclear.<sup>97</sup>

**Spectrum of activity** The antifungal spectrum of flucytosine is extremely narrow and is limited to *Candida* species and *C. neoformans*, although there are some anecdotal recommendations for aspergillosis and chromoblastomycosis.<sup>99</sup> Because resistance to flucytosine may occur at multiple steps in its mode of action, including transport into the cell and deamination to the active compound, flucytosine is only used in combination with other agents, including amphotericin B and fluconazole.<sup>100,101</sup>

**Pharmacokinetics** Flucytosine is a small, very water-soluble molecule and therefore after oral administration it is rapidly and nearly completely absorbed from the intestine.<sup>102</sup> The apparent volume of distribution of flucytosine approximates total body water and the drug distributes well into most body tissues and fluids, including cerebrospinal, vitreous and peritoneal fluids, and inflamed joints.<sup>102-104</sup> Flucytosine is primarily eliminated by the kidneys via glomerular filtration.<sup>102-106</sup> The drug is not secreted into the urine and does not undergo tubular resorption, therefore flucytosine plasma clearance is closely related to creatinine clearance (CrCl).<sup>102,103,105,106</sup> The half-life of flucytosine is approximately 3–4 hours in patients with normal renal function and is significantly prolonged with reductions in renal function.<sup>103</sup> Dosage adjustment is necessary in patients with reduced renal function.<sup>102,105-107</sup> Table 7-5 provides suggested dosage regimens in patients with varying degrees of renal function.

Therapeutic drug monitoring for flucytosine is beneficial and ideally, serum concentrations should be maintained between 25 and 100 µg/ml to minimize toxicity.<sup>108</sup> Although several nomograms exist for dosing flucytosine based on CrCl in patients with renal dysfunction, they are based on serum creatinine measurements and should only be used with chronic renal dysfunction. They should also be used cautiously in elderly

**Table 7-4** Amphotericin B toxicity and its management

Major reactions	Incidence	Management
<b>Infusional adverse reactions</b>		
Fever, chills, rigors, nausea, vomiting, dyspnea, hypoxia, wheezing	Common	Premedicate 30 min before infusion with diphenhydramine (25–50 mg PO q4–6h × 2 doses), acetaminophen (5–50 mg/kg PO q3–4h × 2 doses), meperidine (50–75 mg PO or IM q4–6 h × 2 doses), hydrocortisone (50 mg added to infusion bottle)*
Local phlebitis	Common	Infuse into large veins or through central catheter; if using peripheral vein, infuse slowly and in minimal concentration (0.1 mg/ml), rotate infusion sites and/or add 1000 units of heparin to infusion
Acute anaphylactoid reaction	Rare	Administer test dose; monitor vital signs for 4 h; if acute anaphylactoid reaction develops, discontinue therapy
Hypotension	Rare	Elevate foot of bed; administer 250–500 ml of 0.9% NaCl; discontinue treatment if hypotension is severe or persistent
Hypertension, pain in chest, acute liver failure, thrombocytopenia, vertigo, grand mal seizures, ventricular fibrillation, myocardial infarction, rash	Rare	Specific therapy for each condition
<b>Renal adverse reactions</b>		
Renal insufficiency	Common	Infuse 250–500 ml of 0.9% NaCl 1 h before and 1 h after amphotericin B infusion and/or switch to alternate day; avoid other nephrotoxins† consider non-nephrotoxic azole or echinocandin antifungal agent
Potassium and magnesium losses	Common	Electrolyte replacement
Renal tubular acidosis	Rare	
<b>Constitutional adverse reactions</b>		
Flushing, muscle and joint pain	Common	Supportive care
Normochromic anemia	Rare	Erythropoietin may be indicated; iron of no benefit
Weight loss, weakness	Rare	
*Each agent alone or in combination one or more of the others.		
†Cyclosporine, tacrolimus, cisplatin, aminoglycoside, IV pentamidine, flucytosine.		

patients. During therapy, any necessary dosage adjustments should be based upon plasma concentrations. Lower flucytosine doses (75–100 mg/kg/day) to minimize its toxicity have been advocated and in vitro data suggest that antifungal efficacy would not be compromised by such dosing.<sup>109,110</sup> Hepatic metabolism and protein binding of flucytosine are negligible.<sup>103</sup>

**Toxicity** The primary toxicities of flucytosine are myelosuppression, gastrointestinal intolerance, and hepatic toxicity. The underlying mechanism of myelosuppression associated with flucytosine is unknown. However, the conversion of flucytosine to 5-fluorouracil is believed to be one possible mechanism. Patients treated with flucytosine have detectable



**Table 7-5** Flucytosine dosing in renal impairment

Creatinine clearance (ml/min)	Dose (mg/kg)	Dosing interval
>50	25	Every 6 hours
>25-50	25	Every 12 hours
10-25	25	Daily
<10	12.5	Daily

Notes:  
 1. Monitor renal function and adjust dose accordingly, particularly if other nephrotoxins used (amphotericin B) and if flucytosine serum level is not readily available. Keep peak  $\leq 75$   $\mu\text{g/ml}$  for cryptococcal or candidal meningitis and  $\leq 50$ – $75$   $\mu\text{g/ml}$  for candidemia.  
 2. Monitor blood counts twice weekly and avoid combining flucytosine with myelosuppressive agents.

5-fluorouracil serum concentrations that are comparable to those associated with toxicity in 5-fluorouracil treated patients.<sup>111</sup> The conversion of flucytosine to 5-fluorouracil is thought to occur at least in part via bacterial intraluminal conversion in the intestine with chronic exposure to flucytosine.<sup>111,112</sup> This reaction does not occur in the bloodstream. Myelosuppression occurs more commonly when concentrations exceed 100 mg/l; however, this toxicity may also occur below this threshold concentration.<sup>113</sup> Gastrointestinal side effects (nausea, diarrhea, vomiting, and abdominal pain) occur in an estimated 6% of treated patients.<sup>114</sup> The incidence of flucytosine-associated hepatotoxicity or clinically significant transaminase/phosphatase abnormalities varies from 0% to 41% depending on the definitions employed.<sup>115</sup> The underlying mechanism of flucytosine-associated hepatotoxicity or clinically significant transaminase/phosphatase abnormalities is unknown. This adverse effect often occurs when concentrations exceed 100 mg/l but this toxicity may also occur below this threshold concentration.

## Azoles

Fluconazole, itraconazole, voriconazole, posaconazole (Fig. 7-6)

**Mechanism of action** The azoles exert a fungistatic effect by dose-dependent inhibition of CYP-dependent 14 $\alpha$ -demethylase, which ultimately depletes ergosterol and compromises cell wall integrity. The first clinically useful azoles (clotrimazole, ketoconazole) have excellent activity against *Candida* species. Because of a lack of systemic effect, clotrimazole is used only for mucosal infections. Ketoconazole, the first systemic azole, has largely been supplanted by the other agents in the class and is more commonly used in the developing nations, where its low cost is a major advantage; thus it will not be addressed in this chapter. The systemic azoles developed after ketoconazole include fluconazole, itraconazole, voriconazole and posaconazole.

Among species the systemic triazoles vary in their 14 $\alpha$ -demethylase inhibition, which may in part explain the differences in antifungal activity in this class. The triazoles also

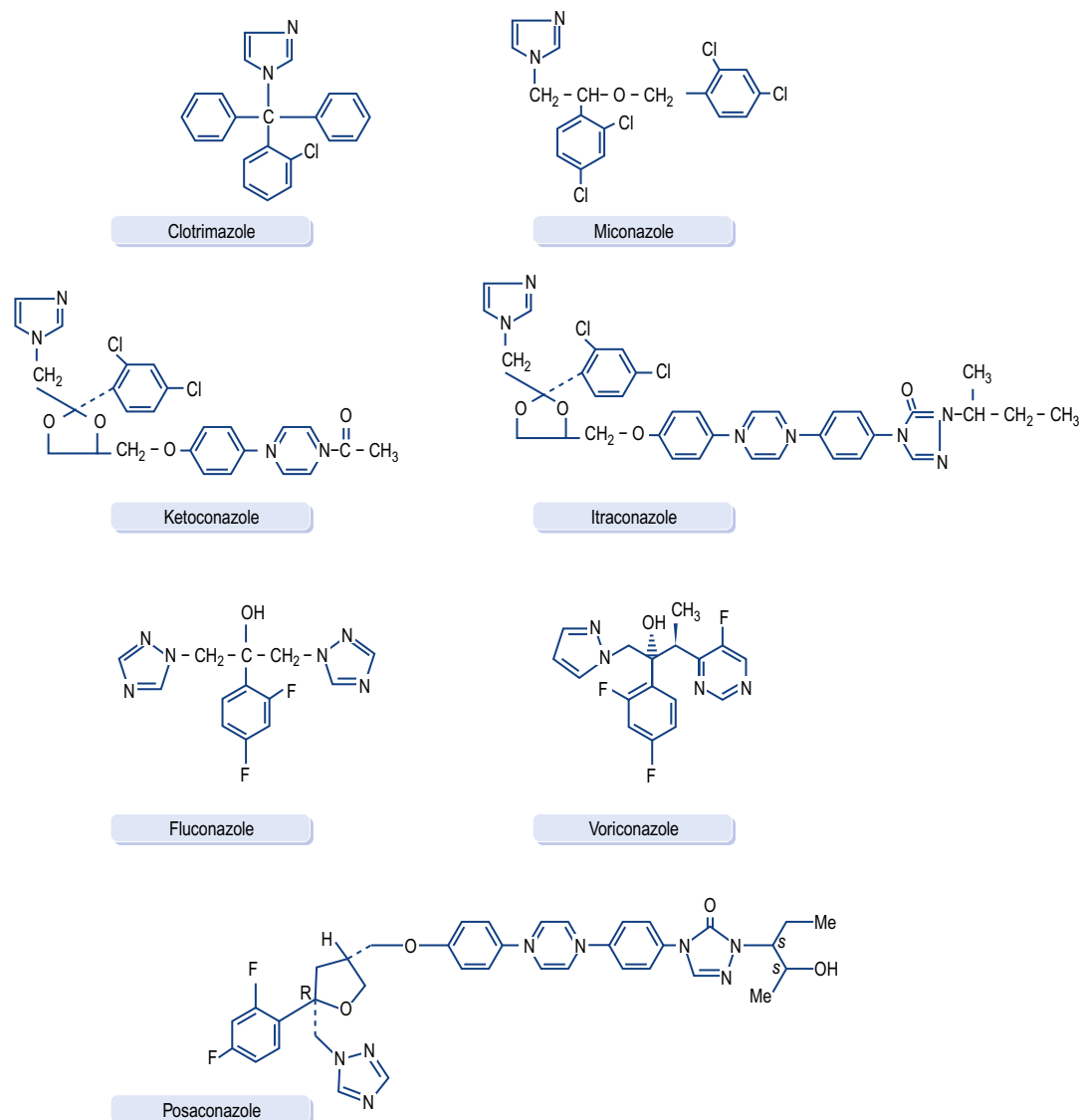
secondarily target other steps in the ergosterol biosynthesis pathway. Depending upon the genus, the affinity for these secondary targets varies among the agents. For example, in fluconazole-susceptible *C. albicans* fluconazole only partially inhibits ergosterol and completely blocks obtusifoliosynthesis, whereas voriconazole completely inhibits both ergosterol and obtusifoliosynthesis.<sup>23</sup> Similarly, in *C. krusei*, both voriconazole and fluconazole completely inhibit obtusifoliosynthesis, but voriconazole inhibits ergosterol synthesis to a greater extent than does fluconazole.<sup>23</sup> Itraconazole and fluconazole may also inhibit 3-ketoreductase, which catalyzes the reduction of the 3-ketosteroid obtusifolione to obtusifoliosynthesis in *C. neoformans*.<sup>19</sup> The resultant accumulation of the 3-ketosteroid obtusifolione and other methylated sterol precursors increases the fragility of the cell membrane. However, in *Histoplasma capsulatum* var. *capsulatum*, it is hypothesized that of these two agents, only itraconazole significantly inhibits 3-ketoreductase.<sup>116</sup> This may explain why itraconazole is more active than fluconazole against this pathogen. All azoles act much more slowly than polyenes. Thus they are used less often than polyenes in treatment of fulminating fungal infections.

**Spectrum of Activity (Table 7-6)** As a class the azoles have a broad spectrum of activity against a variety of yeasts and moulds. However, as this therapeutic class expands, differences in spectrum of activity among the individual agents emerge. As discussed above, the differential spectrum of activity exhibited across the class likely reflects variation in the inhibition of 14 $\alpha$ -demethylase and secondary targets among species.

**Fluconazole** The in vitro activity of fluconazole is generally considered fungistatic and its relatively narrow spectrum of activity is essentially limited to yeasts.<sup>117</sup> Specifically, an 8.5-year global surveillance of susceptibilities of *Candida* species and other yeasts demonstrated that fluconazole is very active against *Candida* species including *C. albicans*, *C. parapsilosis*, *C. tropicalis*, and *C. lusitanae*.<sup>118</sup> However, fluconazole is much less active against other *Candida* spp. *C. krusei* is inherently resistant, and species such as *C. glabrata* and *C. guilliermondii* have reduced susceptibilities to fluconazole.<sup>118</sup> Fluconazole also has activity against *C. neoformans* and *Coccidioides immitis*.<sup>119,120</sup> In general, fluconazole seems to be moderately to severely less active milligram for milligram than other azoles against *H. capsulatum*, *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, *Blastomyces dermatitidis*, and *Penicillium marneffei*.<sup>121-127</sup> Fluconazole has no activity against *Aspergillus* spp., *Fusarium* spp. and the agents of zygomycosis.

**Itraconazole** Itraconazole exerts fungicidal activity against filamentous fungi and some strains of *C. neoformans* and is generally fungistatic against many yeasts.<sup>117</sup> With the exception of *C. glabrata*, itraconazole is moderately to very active against most medically important fluconazole-susceptible and -resistant *Candida* species.<sup>128</sup> However, given the withdrawal of the IV formulation from the marketplace and the variable serum concentrations produced by the oral itraconazole formulations, this agent is not considered a viable option for the treatment of systemic candidiasis.

Itraconazole has modest activity against *C. neoformans*; however, the poor central nervous system penetration of this drug limits its usefulness for treating cryptococcosis.<sup>128</sup> Itraconazole has also excellent in vitro activity against common



**Figure 7-6** Structures of azole antifungals. (A) Clotrimazole. (B) Miconazole. (C) Ketoconazole. (D) Itraconazole. (E) Fluconazole. (F) Voriconazole. (G) Posaconazole.

dimorphic or endemic fungi including *C. immitis*, *H. capsulatum*, *B. dermatitidis*, and *S. schenckii*.<sup>120,129</sup> It has good activity against many *Aspergillus* spp. but it has variable activity against *Fusarium* spp. and very limited activity against the agents of zygomycosis.<sup>128-130</sup> In addition, itraconazole is unique in that hydroxyitraconazole, its primary metabolite in humans, is bioactive. Data suggest that hydroxyitraconazole activity against *C. pseudotropicalis* was nearly twice that of itraconazole but its activity against *A. fumigatus*, *A. terreus*, *C. neoformans*, and *C. immitis* was the same as that of itraconazole.<sup>131</sup> How much hydroxyitraconazole contributes to the activity of itraconazole in vivo is unknown.

**Voriconazole** Voriconazole exerts fungicidal activity against most yeasts and certain opportunistic fungi, and fungicidal activity against some non-*albicans* *Candida* spp. and *C. neoformans*.<sup>117</sup> Voriconazole possesses a very broad spectrum of activity against dermatophytes, yeasts, and

moulds. This agent is active against all *Candida* spp., including fluconazole-resistant *C. albicans*, *C. glabrata*, and *C. krusei*.<sup>118</sup> With the exception of *C. tropicalis*, voriconazole is more active than fluconazole against medically important *Candida* spp.<sup>118</sup> It is very active against other yeasts, including *C. neoformans* and most *Trichosporon* spp., including *T. asahii*, but it is not very active against *T. beigelii*/*T. cutaneum*.<sup>118,119</sup>

Voriconazole exhibits excellent in vitro activity against *Aspergillus* spp. and is highly active against *A. fumigatus*, *A. flavus*, and *A. terreus*.<sup>64</sup> However, over time *A. fumigatus* isolates have become slightly less susceptible to several antifungal agents, including voriconazole.<sup>132</sup> Voriconazole has very potent activity against the dimorphic fungi including *C. immitis*, *H. capsulatum*, *B. dermatitidis*, and *S. schenckii*.<sup>120,129</sup> It is active against many amphotericin-resistant moulds, including certain strains of *Scedosporium apiospermum* (asexual state of *Pseudallescheria boydii*) and *P. boydii*, but it has variable

**Table 7-6** Clinical antifungal activity of the azoles

Species	Fluconazole	Itraconazole	Voriconazole	Posaconazole
<b>Yeasts</b>				
<i>Candida</i> species				
<i>C. albicans</i>	++	++	++	++
Flu/itra resistant	-	-	+	+
<i>C. glabrata</i>	+/-	+/-	+?	+?
<i>C. parapsilosis</i>	++	++	++	++
<i>C. tropicalis</i>	++	++	++	++
<i>C. lusitaniae</i>	++	++	++	++
<i>C. krusei</i>	-	+/-	++	++
<i>C. guilliermondii</i>	+/-	+	++	++
<i>Cryptococcus neoformans</i>	++	++	++	++
<i>Trichosporon asahii</i>	++	++	++	++
<b>Dimorphic fungi</b>				
<i>Coccidioides immitis</i>	+	++	++	++
<i>Histoplasma capsulatum</i>	+/-	++	++	NA
<i>Blastomyces dermatitidis</i>	+/-	++	++	NA
<i>Sporothrix schenckii</i>	+	++	++	NA
<i>Paracoccidioides brasiliensis</i>	+	++	NA	++
<i>Penicillium marneffeii</i>	+	++	++	NA
<b>Moulds</b>				
<i>Aspergillus</i> spp.	-	++	++	++
<i>Fusarium</i> spp.	_*	_*	+/-*	+/-*
Zygomycetes	-	+/-**	-	+**
<i>Scedosporium apiospermum</i>	+/-	++	++	NA
<i>Scedosporium prolificans</i>	-	-	-	-
Phaeohyphomycetes	+	+;++	+;++	+;++
Key: + moderate activity; ++ excellent activity; +/- variable activity; - no clinical activity; flu/itra, fluconazole, itraconazole; NA, insufficient data on clinical activity, probably effective.				
* <i>Fusarium solani</i> is resistant to all azoles; variable susceptibility of other species.				
**Variable susceptibility: species and organism dependent; <i>Rhizopus</i> spp. more susceptible than other species.				



activity against *Fusarium* spp.<sup>64,129</sup> Similar to fluconazole, voriconazole has poor or no activity against the agents of zygomycosis.<sup>64,130</sup>

**Posaconazole** Posaconazole exerts fungicidal activity against non-*albicans* *Candida* species including *C. krusei*, *C. inconspicua* and *C. lusitaniae*, but is fungistatic against *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. guilliermondii* and *C. parapsilosis*.<sup>133</sup> Like voriconazole, posaconazole demonstrates in vitro fungicidal activity against *Aspergillus* spp and *C. neoformans*.<sup>134,135</sup> It is more active than itraconazole and fluconazole against all *Candida* spp. and *C. neoformans*.<sup>136</sup>

In vitro, posaconazole is the most active azole against *Aspergillus* spp. and is highly active against *A. fumigatus*, *A. flavus*, and *A. terreus*.<sup>64</sup> Posaconazole has very potent activity against the dimorphic fungi including *C. immitis*, *H. capsulatum*, *B. dermatitidis*, and *S. schenckii*.<sup>120,129</sup> It also demonstrates variable activity against many amphotericin-resistant moulds, including certain strains of *S. apiospermum* (asexual state of *Pseudallescheria boydii*) and *P. boydii*, but is not active against *Fusarium* spp.<sup>129</sup> Posaconazole has variable activity against the agents of zygomycosis.<sup>64</sup>

**Pharmacokinetics** The systemic azoles differ in chemical properties, which form the basis of the pharmacokinetic differences between the agents and the propensity of this class to interact with other medications. These properties can limit the use of these agents, particularly itraconazole. The pharmacokinetic properties of the commonly used systemic azoles are provided in Table 7-7.

**Fluconazole** The oral formulations of fluconazole are rapidly and nearly completely absorbed. With a bioavailability in excess of 93%, serum concentrations after oral dosing approximate those achieved with IV dosing.<sup>137</sup> The IV formulation should be used only when oral intake is not possible or when oral absorption cannot be assured. In general, administering fluconazole through an enteral feeding tube does not appreciably impact its systemic availability. However, serum concentrations achieved with standard doses administered via an enteral feeding tube may be inadequate to treat *C. glabrata* infections.<sup>138</sup> Fluconazole absorption is not dependent on gastric acidity or the presence of food.<sup>137</sup> Fluconazole binds minimally to plasma proteins (11%), and circulates primarily as free drug. Therefore the drug readily distributes into the CSF and urine, as well as hepatic, renal and CNS tissues.<sup>137</sup> Measurement of fluconazole serum or cerebrospinal fluid concentrations is rarely needed unless there is concern regarding patient compliance, inadequate response to therapy or possible drug-drug interaction.<sup>139</sup>

The chemical properties of fluconazole allow it to circumvent much of the intestinal and hepatic metabolism required by itraconazole or voriconazole for elimination. Increases in fluconazole dosage produce proportional (i.e., linear) changes in serum concentration and systemic exposure. Fluconazole is metabolized by an as yet unidentified CYP and a glucuronidase in the liver to two inactive metabolites. Approximately 91% of an orally administered fluconazole dose is excreted in the urine, mostly (80%) as parent drug, and the two inactive metabolites account for the remaining 11%.<sup>140</sup> Fluconazole undergoes minimal CYP-mediated metabolism but it does weakly inhibit CYP3A4.<sup>141</sup> However, it also more strongly inhibits several other CYP enzymes.<sup>141</sup> Fluconazole binds non-competitively to CYP, and because it circulates largely as free

drug, its ability to inhibit CYP in vitro may not reflect its in vivo inhibitory potential.

Dosage adjustment is necessary in patients with reduced renal function. A 50% dose reduction is recommended for a CrCl between 11 and 50 ml/mn. Patients undergoing hemodialysis should receive one dose after each dialysis session.

**Itraconazole** Itraconazole is available as 100 mg capsules and solubilized in a 40% HP- $\beta$ CD 10 mg/ml solution for oral use. The IV formulation containing HP- $\beta$ CD has been withdrawn from the marketplace. Itraconazole absorption from the capsule form is slow and incomplete, and the drug undergoes significant “first-pass” metabolism in the intestine and liver before reaching the systemic circulation. In capsule form, absorption is variable and itraconazole is better absorbed under acidic gastric conditions or in the fed state.<sup>142</sup> In contrast, HP- $\beta$ CD significantly enhances the solubility of itraconazole. The oral solution requires no dissolution so its absorption is not influenced by gastric pH and is rapid.<sup>143</sup> Thus, high concentrations of itraconazole are delivered to the intestinal epithelium, which may cause transient saturation of intestinal CYP3A4.<sup>144,145</sup> The oral solution undergoes less “first-pass” metabolism, and therefore produces higher and more consistent serum concentrations. In this form, itraconazole is better absorbed in the fasting than the fed state.<sup>144</sup> Higher peak plasma concentrations of itraconazole and its primary metabolite, hydroxyitraconazole, are achieved more rapidly following administration in the fasted state compared to non-fasting conditions.<sup>144,145</sup> Compared to the capsule, the oral solution produces a pharmacokinetic profile with less inter- and inpatient variability.<sup>146</sup> Although the oral solution is optimally absorbed under fasting conditions, even in the fed state it produces higher serum concentrations than the capsule. The absolute bioavailability of the oral solution is higher than that of the capsule, but the two formulations are considered bioequivalent.<sup>142,146</sup>

Itraconazole is highly lipophilic. In the serum it is highly bound (99.8%) to albumin and consequently the unbound concentrations in body fluids (i.e., CSF, saliva, urine) are very low.<sup>147</sup> Itraconazole distributes widely throughout the body and has high affinity for tissues (i.e., vaginal mucosa, horny layer of nails, etc.).<sup>147</sup> It can persist in these tissues long after the serum concentrations are undetectable.<sup>147</sup> Increases in itraconazole dosage produce disproportional (i.e., non-linear) changes in drug levels. Itraconazole is extensively metabolized and several metabolites are sequentially formed only by CYP3A4, including hydroxyitraconazole, ketoitraconazole, and N-desalkyl-itraconazole.<sup>148</sup> The principal metabolite, hydroxyitraconazole, is formed primarily during gut wall metabolism and is bioactive.<sup>142,148</sup>

**Voriconazole** Voriconazole is available as an IV and oral formulation. The IV formulation consists of a powder for reconstitution containing 200 mg voriconazole solubilized with sulfobutyl ether  $\beta$ -cyclodextrin (SE- $\beta$ CD). When reconstituted, the final solution contains 10 mg/ml. The oral tablets contain either 50 or 200 mg of voriconazole. Voriconazole dissolution is not affected by altered gastric pH. Following oral dosing, voriconazole absorption is rapid and nearly complete, with a relative bioavailability approaching 90%.<sup>149</sup> Peak serum concentrations are achieved within 2 hours of oral dosing. Voriconazole is moderately bound to plasma proteins and is widely distributed throughout the body. In case reports, CSF concentrations

**Table 7-7** Summary of oral azole dosing and pharmacokinetics

	Fluconazole	Itraconazole		Voriconazole	Posaconazole
		Cap	Soln		
Absorption					
Rate	Rapid (1–3 h)	Slow	Rapid	Rapid	Slow
Bioavailability (%)	>93	30	55	96	–
Food effect	No	Yes	Yes	Yes	Yes
Increase ( )/ Decrease ( )	None				
Distribution					
Protein binding (%)	Minimal (<10)	Significant (99.8)		Moderate (60)	Significant (>95)
VD (L/kg)	0.7–0.8	10.7		4.6	Very large
CNS/CSF penetration	60–80%	<1%		>50%	–
Metabolism					
CYP substrate	Yes (moderate)	Yes (major)		Yes (major)	Yes (mild)
Isoform (s)	2C9/19; 3A4	3A4		2C19; 3A4; 2C9	3A4
CYP inhibitor	Yes	Yes		Yes	Yes
Isoform (s)	2C9/19; 3A4	3A4		2C9; 2C19; 3A4	3A4
Phase II substrate	No	No		–	Yes
Isoform	–	–		–	UGT1A4
Phase II inhibitor	Yes	No		–	–
Isoform	UGT2B7	–		–	–
Other pathway(s)	–	–		–	–
Transporters					
P-gp substrate	No	Yes		No	Yes
P-gp inhibitor	No	Yes		No	Yes
Other transporters	–	–		–	–
Transport protein	–	BCRP (I)		–	–
Elimination					
Urine	Yes (89%)	No (<1%)		No (<5%)	No (<14%)
Bile/Feces	No	Yes		Yes	Yes (77%)
Key: – unknown/not applicable; Cap, capsule; Soln, solution; VD, volume of distribution; UGT, UDP-glucuronosyltransferase; COMT, catechol-O-methyltransferase; OATP, organic anion transporting polypeptide; BCRP (I), breast cancer resistance protein inhibitor.					

achieved with standard dosing have been approximately 30–60% of plasma concentrations.<sup>149,150</sup> Voriconazole concentrations in brain tissue are higher than those in the CSF.<sup>151</sup>

In adults, increases in voriconazole dosage produce disproportional (i.e., non-linear) changes in drug levels. In contrast, increases in voriconazole dosage in children given low-dose voriconazole produce proportional (i.e. linear) changes in drug levels. Moreover, higher doses are required in children.<sup>149</sup> Voriconazole is metabolized by several CYP enzymes including CYP2C19, 2C9, and 3A4.<sup>152</sup> The primary voriconazole metabolite in man is formed by CYP2C19, CYP3A4, and, to some extent, CYP2C9.<sup>152</sup> Both CYP2C19 and CYP2C9 exhibit genetic polymorphisms that add to the complexity of voriconazole pharmacokinetics. Therefore age-related differences are probably due to the saturation of CYP enzymes in adults, polymorphisms or age-related differences in CYP expression. However, at higher dosages data suggest that voriconazole exhibits non-linear pharmacokinetics in children.<sup>153</sup>

Because clearance of SE- $\beta$ CD excipient is reduced fourfold in moderate to severe renal impairment (CrCl 30–50 ml/min), it is recommended that the IV route be avoided if CrCl <50 ml/min unless the benefits outweigh the risk. No dose reduction is needed when using the oral formulation. It is also recommended that the maintenance dose of voriconazole be reduced by 50% in the presence of moderate hepatic cirrhosis; however, a standard loading dose should still be used in this setting.

**Posaconazole** Posaconazole, a highly lipophilic weak base, is chemically similar to itraconazole. Posaconazole is available only as an oral suspension. It is systemically available and eliminated slowly, with consistent pharmacokinetic values at specific dose levels following single and multiple doses given to healthy volunteers as tablets or the marketed oral suspension.<sup>154–157</sup> Healthy volunteer studies indicate that posaconazole systemic availability is optimized with the oral suspension and that increases in dosage up to 800 mg/day produce proportional (i.e., linear) changes in drug levels. Posaconazole oral suspension administered in the fed state, particularly after a high-fat meal, provides optimal oral drug exposure.<sup>154</sup> Based on drug exposure and maximum serum concentration values, relative oral bioavailability estimates are fourfold and 2.6-fold greater following administration of the posaconazole oral suspension with a high-fat and non-fat meal, respectively.<sup>154</sup>

The oral administration of 600–800 mg/day in divided doses (200 mg 3 times/day or 400 mg twice/day) maximizes posaconazole exposure.<sup>158</sup>

In neutropenic hematopoietic stem cell transplant recipients, increasing dosing frequency and doses up to 800 mg/day produced dose-related but less than dose increases in maximum serum concentrations and exposure.<sup>159</sup> The reasons for this finding are unclear, but likely related to the poor nutritional intake, vomiting, and diarrhea common to this population. While mucositis reduced exposure, the effect was not significant and was lessened with increasing total dose up to 800 mg/day and administering it in divided doses. From a pharmacokinetic standpoint there is no benefit to administering total daily doses in excess of 800 mg singly or in divided doses.<sup>158</sup> Posaconazole is metabolized less than itraconazole or voriconazole.<sup>157</sup> In contrast to itraconazole and voriconazole, posaconazole is only minimally metabolized by CYP. Most posaconazole metabolites are glucuronide conjugates formed by uridine diphosphate glucuronosyltransferase (UGT) pathways.<sup>160</sup>

**Toxicity** The azoles are a relatively safe class of drugs and are associated with few serious adverse effects. The advent of fluconazole and subsequent agents greatly improved the safety of this class. All the azoles are associated with gastrointestinal intolerance, transient transaminitis, hepatic toxicity, rashes, dizziness and psychosis.<sup>161,162</sup> Gastrointestinal symptoms (nausea, vomiting, and diarrhea) are the most common side effects associated with this class, particularly with the oral itraconazole solution.<sup>161</sup> These effects are usually encountered with high doses of these compounds, but the symptoms are rarely severe enough to necessitate discontinuation of therapy.

Clinically significant transaminitis occurs commonly with all azoles. In general, patients experiencing azole-associated transaminase abnormalities are asymptomatic, but these increases can, on rare occasions, evolve into fatal drug-induced hepatitis.<sup>161</sup> Consequently, clinicians should obtain baseline liver function tests prior to starting azole therapy. Moreover, patients receiving azoles should periodically be monitored for evidence of drug-induced hepatitis.<sup>161</sup>

The azoles can also produce allergic skin rashes that are generally mild and subside with discontinuation of the drug. The azoles produce teratogenic effects in mice and therefore their use should be avoided in pregnancy (Category C).

**Fluconazole** Fluconazole is the safest azole and doses 4–5 times in excess of the recommended daily dose have been well tolerated. Gastrointestinal symptoms associated with fluconazole use rarely occur and when they do, they are frequently considered mild. Fluconazole may also produce transient transaminase abnormalities, but progression to severe drug-induced hepatitis is exceedingly rare. Fluconazole does not inhibit human steroidogenesis. Nonetheless, it has produced alopecia in up to 20% of patients receiving at least 400 mg/day for more than 2 months.<sup>163</sup> This alopecia is apparently reversible and resolves within 6 months of stopping therapy or reducing the dose by 50%.<sup>163</sup>

**Itraconazole** When administered in dosages of 400 mg/day or less, itraconazole generally produces little toxicity. However, the incidence of adverse effects increases with prolonged courses of at least 400 mg/day, but rarely does the toxicity require drug discontinuation.<sup>164</sup> Like all azoles, transient transaminase abnormalities and gastrointestinal adverse effects, particularly nausea, abdominal pain and diarrhea, are common with itraconazole administration.<sup>164</sup> These symptoms are generally described as mild by patients receiving the capsule form. However, with the oral solution they occur more frequently and are more severe.<sup>165</sup> In clinical trials in patients with AIDS who received the oral solution, gastrointestinal adverse events were so severe that 8–10% of patients discontinued the drug.<sup>165</sup> The diarrhea associated with the oral solution is generally attributed to an osmotic effect of the HP- $\beta$ CD in the GI tract.<sup>165</sup> Itraconazole on rare occasion can produce yet to be explained life-threatening reactions (liver failure, CHF). Even though these adverse effects are very rare, their risk of occurrence should be considered when using itraconazole to treat non-life threatening infections of the skin and nail beds. When used in recommended dosages, itraconazole has little or no inhibitory effect on human steroidogenesis. However, patients may experience a mineralocorticoid excess syndrome manifested by hypokalemia, hypertension, and edema with doses in excess of 400 mg/day, especially with protracted courses.<sup>161,162</sup>



**Voriconazole** Voriconazole is generally well tolerated. In addition to the gastrointestinal and dermatologic adverse effects seen with other azoles, voriconazole produces visual disturbances and clinically significant transaminase abnormalities in approximately 30% and 12.7% of patients, respectively.<sup>166</sup> These adverse effects are typically benign and rarely lead to discontinuation of therapy. The visual disturbances are acute and include changes in color discrimination, blurred vision, photophobia, and the appearance of bright spots.<sup>162</sup> These disturbances generally require little or no therapy and diminish shortly into a course of therapy without producing lasting retinal damage.<sup>152,162</sup> The underlying mechanism for this adverse effect is unknown.

Like the other azoles, the transaminase abnormalities associated with voriconazole are common and mild but on rare occasions, life-threatening hepatitis has been described.<sup>166</sup> Whether voriconazole is more hepatotoxic than other azoles or whether this adverse effect is correlated with voriconazole dose or serum concentrations is debatable. Data suggest that transaminase abnormalities increase with voriconazole dose.<sup>167,168</sup> Furthermore, transaminase abnormalities or liver failure developed in six of 22 (27%) patients with invasive aspergillosis who had voriconazole serum concentrations greater than 6 µg/ml.<sup>169</sup> However, in phase 2 and 3 clinical trials, voriconazole frequently produced transaminitis, yet the majority of cases were mild to moderate

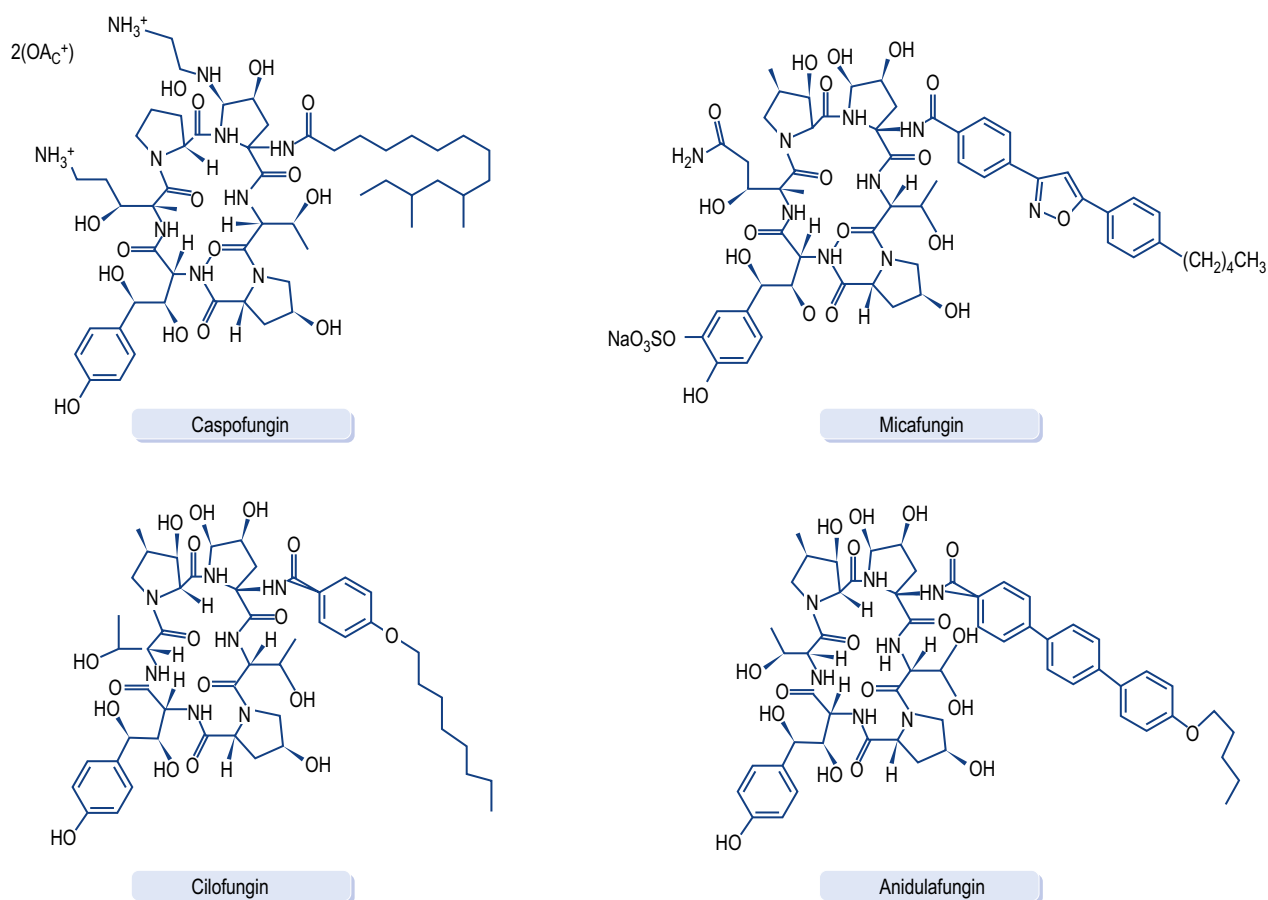
in severity and rarely (≈3%) resulted in drug discontinuation.<sup>170</sup> A retrospective longitudinal logistic regression analysis provides compelling data suggesting that factors other than elevated serum drug concentrations contribute to transaminase abnormalities in patients receiving voriconazole.<sup>166</sup> In voriconazole clinical studies allergic skin rashes occurred in approximately 20% of patients.<sup>162</sup> These reactions were generally considered mild in severity and did not interrupt therapy.

**Posaconazole** Compared to other azoles, there is much less clinical experience with this agent. Consequently its safety profile may not be fully realized. Nonetheless, to date the common adverse effects associated with posaconazole use have been similar to those observed with other agents in the class (i.e., gastrointestinal, transient transaminase abnormalities).<sup>162</sup>

## Echinocandins

Caspofungin, micafungin, anidulafungin (Fig. 7-7)

**Mechanism of action** The echinocandins are synthetic lipopeptides that are derived from fermentation products from several different fungi. These compounds disrupt cell wall synthesis by inhibiting a novel target, β1,3-D-glucan synthase, which blocks β1,3-D-glucan synthesis.<sup>171</sup> This enzyme is present in most fungal pathogens but is not present



**Figure 7-7** Structures of echinocandins. (A) Caspofungin. (B) Micafungin. (C) Cilofungin (D) Anidulafungin.

in mammalian cells and its inhibition ultimately produces osmotic lysis of the cell.  $\beta$ 1,3-D-glucans are critical components of most fungal cell walls and provide morphology and structural integrity.

**Spectrum of activity** These agents bind rapidly and irreversibly to  $\beta$ 1,3-D-glucan synthase and cause rapid death in certain pathogens. The echinocandins possess a narrow antifungal spectrum that is restricted to *Candida* spp. and *Aspergillus* spp. and there is little difference among the individual drugs. All the echinocandins exert fungicidal activity

against *Candida* spp.; however, in *Aspergillus* spp. these compounds do not usually cause complete inhibition of growth but instead induce abnormal morphologic hyphal growth.<sup>172</sup> Therefore these agents are considered to be fungistatic against *Aspergillus* spp.

In vitro the echinocandins are highly active against *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. dubliniensis*, and *C. krusei*.<sup>173-176</sup> They are slightly less active against *C. parapsilosis*, *C. guilliermondii*, and *C. lusitaniae* and MIC values are typically higher for these pathogens than other

**Table 7-8** Summary of echinocandin pharmacokinetics

	Caspofungin	Micafungin	Anidulafungin
<b>Distribution</b>			
Protein binding (%)	Significant (97)	Significant (99)	Significant (84)
VD (l/kg)	0.15	0.4	0.6
CNS/CSF penetration	Minimal	Minimal	–
<b>Metabolism</b>			
CYP substrate	No	No	No
Isoform(s)	–	–	–
CYP inhibitor	No	No	No
Isoform(s)	–	–	–
Phase II substrate	No	No	No
Isoform	–	–	–
Phase II inhibitor	No	No	No
Isoform	–	–	–
Other pathway(s)	N-acetylation Peptide hydrolysis	Arylsulfatase COMT hydroxylation	Chemical degradation
<b>Transporters</b>			
P-gp substrate	No*	No	No
P-gp inhibitor	No*	No	No
Other transporters	Yes	–	–
Transport protein	OATP1B1	–	–
<b>Elimination</b>			
Urine	Yes (41%)	No (<1%)	No (<1%)
Bile/Feces	Yes (35%)	Yes (71%)	No (<10%)
* , only at concentrations in excess of those achieved clinically Key: –, unknown/not applicable; VD, volume of distribution; COMT, catechol-O-methyltransferase; OATP, organic anion transporting polypeptide.			

*Candida* spp.<sup>173-176</sup> In general, the echinocandins are very active against *A. flavus*, *A. fumigatus*, and *A. terreus*.<sup>177-180</sup> Inhibition of growth is observed at very low concentrations, but the in vitro activity can be influenced by inoculum size and media composition. Therefore there may be subtle differences in the in vitro activity of the individual echinocandins against *Aspergillus* spp. that currently can be difficult to discern.<sup>177-180</sup> The echinocandins are also active against *Pneumocystis jirovecii*. In vitro this class also has modest activity against dimorphic fungi including *C. immitis*, *H. capsulatum*, and *B. dermatitidis*, but this activity is not considered to be clinically useful. The echinocandins have little or no activity against *C. neoformans*, *Trichosporon* spp., *Fusarium* spp. or any agents of zygomycosis.<sup>181</sup>

**Pharmacokinetics** Due to their molecular size all the echinocandins are available only as IV formulations. The echinocandins differ little in their chemical properties; thus, following IV administration they demonstrate similar pharmacokinetic behavior. The pharmacokinetic properties of the echinocandins are summarized in Table 7-8. The primary difference between the agents lies in how they are metabolized and how they distribute throughout the body, which influences their elimination half-lives. Increases in the dosage of all the echinocandins produce proportional (i.e., linear) changes in serum concentration and systemic exposure. The echinocandins are not appreciably metabolized by CYP.

**Caspofungin** The distribution of caspofungin is complex and involves several distinct phases. Following IV administration, caspofungin is extensively bound to albumin and thus initially it largely distributes only to the plasma and extracellular fluid.<sup>182</sup> Subsequently it slowly distributes into the tissue, primarily the liver, via an active transport process involving the organic anion transport proteins (OATP), specifically OATP1B1.<sup>182,183</sup> This is a very slow process that influences the subsequent elimination half-life of the drug.<sup>182</sup> Caspofungin is slowly metabolized in the liver via *N*-acetylation and peptide hydrolysis to inactive metabolites, which are excreted in the bile and feces.<sup>184</sup>

In the presence of moderate hepatic impairment, the maintenance dose of caspofungin should be reduced to 35 mg per day. However, a standard loading dose of 70 mg should still be used in this setting.

**Micafungin** The distribution and metabolism of micafungin are not completely understood. Following IV administration, micafungin is extensively bound to plasma proteins, primarily albumin, and, to a lesser extent, to  $\alpha$ 1-acid glycoprotein and its apparent volume of distribution is larger than that of caspofungin. Micafungin is metabolized to several metabolites that are formed by hepatic reactions catalyzed by arylsulfatase, catechol-O-methyltransferase, and to a minor extent  $\omega$ 1-hydroxylation via CYP.<sup>185-187</sup> Very little (<1%) of a micafungin dose is eliminated as unchanged drug in the urine. Approximately 70% of an administered dose is eliminated as parent drug and metabolite(s) in feces.<sup>185-187</sup>

**Anidulafungin** The distribution and metabolism of anidulafungin are not fully understood. Following IV administration, anidulafungin is less bound to plasma proteins, has a larger volume of distribution and achieves lower peak serum concentrations than caspofungin or micafungin.<sup>186</sup> Anidulafungin primarily undergoes slow non-enzymatic degradation in the plasma to a peptide breakdown product, which is enzymatically

degraded and excreted in the feces and bile. Very little anidulafungin is excreted in the feces or urine as unchanged drug.<sup>188</sup>

**Toxicity** The echinocandins are well tolerated and their use is associated with very few significant adverse effects. Common adverse effects include irritation at the injection site, phlebitis, and transient transaminase abnormalities. Other less common non-specific adverse effects include nausea, vomiting, diarrhea, fever and headache.

## Allylamines

### Terbinafine

**Mechanism of action** Terbinafine reversibly inhibits squalene epoxidase, an enzyme that acts early in the ergosterol synthesis pathway. This inhibition produces both the fungistatic and fungicidal effects on fungal cells.

**Spectrum of activity** In vitro, terbinafine demonstrates excellent fungicidal activity against many dermatophytes including *Trichophyton rubrum*, *T. mentagrophytes*, *T. tonsurans*, *Microsporum canis* and *Epidermophyton floccosum*.<sup>189</sup> However, terbinafine demonstrates variable and somewhat poor in vitro activity against many yeasts. It generally demonstrates fungicidal activity against *C. parapsilosis* but it is fungistatic against *C. albicans* and other *Candida* spp.<sup>189</sup> The in vitro spectrum of activity also includes *Aspergillus* spp., some dimorphic fungi, *S. schenckii*, and others. Initial animal studies in mice showed no activity in vivo against systemic pathogens, and the drug was abandoned for these indications.

**Pharmacokinetics** Terbinafine is well absorbed after oral administration, but it undergoes significant “first-pass” metabolism and its resulting apparent bioavailability is only 45%.<sup>190,191</sup> It is metabolized to ten metabolites by at least seven different CYP enzymes, the most important apparently being CYP2C9, CYP1A2, and CYP3A4.<sup>192</sup> Terbinafine binds extensively to plasma proteins.<sup>193</sup> Of note, it distributes extensively to poorly perfused tissues (i.e., skin and ungual bed).<sup>189</sup> Terbinafine is extensively metabolized by the liver and 15 metabolites have been identified.<sup>189</sup>

**Toxicity** Terbinafine produces few adverse reactions, including perversion of taste perception and occasionally abnormal liver function.

## Antifungal drug–drug interactions

### Mechanisms of antifungal drug–drug Interactions

#### Amphotericin B formulations

Drug interactions associated with the amphotericin B formulations result from the amphotericin B-associated nephrotoxicity and electrolyte disturbances that interfere with the ability to use other agents. Decreasing the risk of amphotericin B-associated toxicity (i.e., prehydration with normal saline, using lipid amphotericin B formulations or other antifungal agent in patients receiving other nephrotoxins) is key to avoiding the interactions. If possible, monitoring plasma concentrations of renally eliminated drugs with a narrow therapeutic index and serum electrolytes will also help minimize any risks.

Agents that cause additive or synergistic nephrotoxicity with amphotericin B include immunosuppressants (cyclosporine



tacrolimus), antiinfective agents (aminoglycosides, cidofovir, foscarnet, pentamidine IV and polymyxins B and E), antineoplastic agents (cisplatin, nitrosoureas, others) and interleukin-2. In addition, attention should be paid to renally excreted agents whose clearance is reduced by amphotericin-related nephrotoxicity. Increased toxicity of these agents may result from their delayed clearance. Agents commonly used in patients receiving amphotericin B include many renally excreted antineoplastic agents (monitor for individual agent toxicity), flucytosine or ganciclovir (increased hematologic toxicity). Finally, the concomitant administration of amphotericin B and some agents (e.g., foscarnet) may cause additive or synergistic electrolyte abnormalities. Amphotericin B also induces hypokalemia which may increase digoxin toxicity. Finally, because of the risk of severe arrhythmias, it would be preferable to avoid using amphotericin B with drugs known to induce torsade de pointes (amiodarone, bepridil, disopyramide, erythromycin, pentamidine, quinidine and sotalol).

### Fluorinated pyrimidine analog

Drug interactions associated with flucytosine result from renal dysfunction produced by concomitantly administered nephrotoxic drugs (i.e., amphotericin B or aminoglycosides), which ultimately reduces flucytosine elimination and increases its serum concentrations. Properly managing a patient's renal function and adjusting the flucytosine dose accordingly is key to avoiding interactions with this agent.

### Azoles

Due to their chemical properties, drug interactions associated with the azoles result from several different mechanisms. All azoles are weak bases and at elevated pH values, weakly basic compounds dissolve more slowly. Therefore, the absorption of the capsule form of itraconazole is influenced by alterations in gastric pH. Many of the azoles are lipophilic and thus they are subjected to interactions involving their biotransformation and disposition. Fluconazole is hydrophilic and is highly soluble in water and therefore, compared to the other azoles, it requires much less biotransformation to be eliminated from the body.<sup>194</sup> Itraconazole, voriconazole, and posaconazole are highly lipophilic and have limited aqueous solubility.<sup>159,194</sup> Therefore, these azoles must undergo extensive enzymatic conversion to more polar metabolites in order to be eliminated from the body.

**Interactions with biotransformation enzymes** As discussed above, all azoles are CYP substrates but the degree to which they are metabolized by CYP proteins differs. This CYP-mediated metabolism is carried out primarily in the liver and intestine. The azoles and the itraconazole metabolites are potent competitive or non-competitive inhibitors of CYP, although they differ in their respective affinities for these enzymes.<sup>194,195</sup> The azoles predominantly inhibit CYP3A4, which is the most common CYP involved in drug metabolism. Fluconazole binds non-competitively to CYP and inhibits several CYP enzymes. Fluconazole strongly inhibits CYP2C9 and CYP2C19 but only weakly inhibits CYP3A4.<sup>196</sup> However, because it binds non-competitively and circulates in the body primarily as unbound drug, its *in vitro* inhibitory potential may not predict its inhibition potential *in vivo*. Itraconazole and its metabolites potently inhibit only CYP3A4. Because itraconazole is a potent inhibitor of this enzyme and CYP3A4 is solely responsible for itraconazole clearance, it is not surprising that this drug causes many inhibitory interactions with other drugs cleared by CYP3A4.<sup>195</sup>

Like fluconazole, voriconazole inhibits several CYP enzymes, including CYP2C9, CYP2C19, and CYP3A4.<sup>197</sup> Like itraconazole, posaconazole inhibits only CYP3A4.<sup>198</sup>

**Interactions with transport proteins** Transport proteins expressed throughout the body facilitate the absorption, uptake or efflux of many drugs or xenobiotics. P-glycoprotein (P-gp), a large transmembrane efflux protein, is extensively co-localized with CYP3A in the intestine, liver, kidney, and the cells of the blood-brain barrier.<sup>199</sup> This protein also transports many CYP3A4 substrates. Among the azoles, itraconazole, posaconazole and possibly fluconazole are P-gp substrates.<sup>200-203</sup> However, only itraconazole inhibits P-gp.<sup>204</sup>

Although P-gp is the most characterized transport protein, there are many other transport proteins (i.e., multi-drug resistance (-associated) proteins, OATPs, organic cation transporters, organic anion transporters, and breast cancer resistance protein) that contribute to drug disposition.<sup>205</sup> Many of these transporters are distributed in tissue throughout the body and function much like P-gp.<sup>205</sup> These proteins transport a variety of medicines but their interactions with the azoles have not been well characterized.

### Echinocandins

As a class the echinocandins interact very little, if at all, with CYP or P-gp. Therefore, to date their use has been associated with very few significant drug interactions. Caspofungin is a substrate of the transport protein OATP1B1.<sup>183</sup> This protein is involved in the slow distribution of caspofungin into tissue and it is believed that interactions with this protein by other drugs may cause drug interactions with caspofungin.<sup>183</sup>

### Terbinafine

Although terbinafine is metabolized by at least seven different CYP enzymes, it does not inhibit most CYP enzymes at clinically relevant concentrations.<sup>192</sup> However, terbinafine and at least two of its metabolites do inhibit CYP2D6.<sup>192</sup> Unlike CYP3A4, this enzyme does not represent a large portion of hepatic CYP and it is involved in the metabolism of only a few therapeutic drug classes.

## Clinically significant antifungal drug-drug interactions that impact other agents

### Azoles (Tables 7-9 to 7-13)

Drug interactions involving fluconazole that impact other drugs are summarized in Tables 7-9 and 7-13. Clinically relevant interactions with fluconazole result primarily from CYP interactions. Drugs that produce clinically significant CYP-mediated interactions with fluconazole include several benzodiazepines and anxiolytics, calcineurin inhibitors, phenytoin, and warfarin.<sup>194,200</sup> One of the most important interactions occurs with warfarin. The interaction between fluconazole and warfarin is highly predictable. Warfarin is a racemic compound and its pharmacologic activity is produced by the *S*-enantiomer. This enantiomer is metabolized by CYP2C9. Fluconazole inhibits the metabolism of *S*-warfarin by approximately 70% and produces a 38% increase in the international normalized ratio (INR) in patients previously stabilized on warfarin.<sup>141</sup> This combination should be avoided.

**Table 7-9** Clinically significant interactions with fluconazole

Drug	Comments and Recommended Actions
Fluconazole affects drug: monitor for clinical toxicity and for toxic-range blood levels. Adjust dose	
Calcineurin inhibitor	
Cyclosporine	↑ C <sub>min</sub> and ↓ clearance of all three agents via inhibition of hepatic and perhaps enteric CYP3A. ↓ calcineurin inhibitor dose and monitor level.
Benzodiazepines	
Midazolam	↑ midazolam and triazolam C <sub>max</sub> , exposure, t <sub>1/2</sub> , and bioavailability ~ 2-fold; involves hepatic and perhaps enteric CYP3A. ↑ sedation. Monitor sedation and ↓ midazolam dose.
Triazolam	
Miscellaneous drugs	
Phenytoin	↑ phenytoin C <sub>min</sub> ~1.25-fold and phenytoin exposure by 75% via dose-dependent inhibition of hepatic CYP3A4. Monitor phenytoin levels.
Warfarin	Inhibits primary pathway of warfarin biotransformation by 70% via inhibition of hepatic CYP2C9 by fluconazole. ↑ INR and risk of bleeding with warfarin. Monitor INR.
Alfentanil	↑ alfentanil exposure and t <sub>1/2</sub> ~2-fold; ↓ alfentanil clearance by ~50% and steady-state volume of distribution by ~20% via inhibition of hepatic CYP3A4.
Ibuprofen	↑ S-(+)-ibuprofen exposure by 183%; ↑ peak concentration by 116%; ↑ t <sub>1/2</sub> .
Sulfonylureas	↑ AUC and t <sub>1/2</sub> via inhibition of CYP2C9 by fluconazole
Drug affects Fluconazole: monitor for antifungal response	
Phenytoin	Phenytoin ↓ fluconazole concentrations via induction of CYP 3A4.
Rifampin/Rifabutin	Antibiotics ↓ fluconazole concentrations via induction of CYP 3A4.
See also Table 7-13. Key: C <sub>max</sub> , peak concentration; C <sub>min</sub> , trough concentration; CYP, cytochrome pathway; t <sub>1/2</sub> , half-life.	

Drug interactions involving itraconazole that impact other drugs are summarized in Tables 7-10 and 7-13. Clinically relevant interactions with itraconazole result from several mechanisms, including alterations in gastric pH, CYP3A4 and transport protein interactions. Drugs that can produce clinically significant interactions with itraconazole include gastric pH modifiers (i.e.,  $H_2$ -receptor antagonists, proton pump inhibitors, antacids), CYP3A4 substrates (i.e., many of the statins, several benzodiazepines and anxiolytics, the calcineurin inhibitors, corticosteroids, calcium channel blockers of the dihydropyridine class) and P-gp substrates (digoxin, quinidine and the vinca alkaloids).<sup>194,200,206</sup> Although alterations in gastric pH significantly reduce the absorption of the itraconazole capsule, such changes do not affect the absorption of the oral solution.<sup>143</sup>

Due to the extent of interactions between itraconazole and several CYP3A4 substrates, including midazolam, triazolam and dihydropyridine calcium channel blockers, these agents should be avoided in patients receiving itraconazole.<sup>194,200,206</sup> Similarly, itraconazole use in patients receiving vinca alkaloids (i.e., vincristine, vinblastine) should be avoided.

Drug interactions involving voriconazole that impact other drugs are summarized in Tables 7-11 and 7-13. Clinically relevant interactions with voriconazole result primarily from interactions with CYP2C19, CYP2C9, and CYP3A4. Drugs that can produce clinically significant interactions with voriconazole include CYP2C19, CYP2C9, and CYP3A4 substrates (i.e., immunosuppressive agents such as cyclosporine and tacrolimus, certain benzodiazepines, analgesics, warfarin, phenytoin, and perhaps statins and corticosteroids).<sup>194,200,206</sup> Although many interactions with voriconazole may be managed, the extent of interactions between voriconazole and the immunosuppressants tacrolimus or sirolimus may be too significant to overcome. If possible, these combinations, particularly voriconazole and sirolimus, should be avoided.<sup>194,200,206</sup>

Clinically relevant interactions with posaconazole result primarily from interactions with CYP3A4 and Phase II enzymes in the UGT pathways. Because experience with posaconazole is relatively limited, there are few published data from appropriately controlled studies investigating drug interactions involving this agent. However, drugs that can produce significant interactions with posaconazole (Table 7-12) include rifabutin, calcineurin inhibitors, cimetidine, glipizide and phenytoin.<sup>207-209</sup>

As described above, all azoles interact significantly with the calcineurin inhibitors and benzodiazepines such as midazolam and triazolam. Using an azole with an agent in these drug classes at times may be necessary. For example, the use of an azole with a calcineurin inhibitor in an immunosuppressed patient at high risk for systemic mycoses may be unavoidable. Recommended dosage adjustments and expert opinion-derived guidelines exist for managing interactions in this population but while these resources may serve as a useful starting point, clinicians should not rely solely on them to manage these interactions. Instead, clinicians should monitor blood concentrations of the calcineurin inhibitors before, during, and after azole use and base any dosage adjustment on the objective results of these blood concentration data.<sup>200,210</sup>

Table 7-13 provides a more comprehensive list of the drug–drug interactions involving the antifungal azoles.

**Table 7-10** Clinically significant interactions with itraconazole (ITRA)

Drug	Comments and recommended actions
<b>ITRA affects drug: monitor for clinical toxicity and for toxic-range blood levels. Adjust dose</b>	
<b>Calcineurin inhibitors</b>	
Cyclosporine Tacrolimus Sirolimus	↑ $C_{min}$ (cyclosporine, tacrolimus) via inhibition of hepatic CYP3A4 and perhaps enteric P-gp. ↑ AUC, $C_{max}$ (sirolimus) via inhibition of hepatic CYP3A4 and perhaps enteric P-gp. When starting ITRA, reduce cyclosporine and tacrolimus dose by 50%. Closely monitor calcineurin inhibitor blood levels and increase dose when discontinuing ITRA. Limited information on dose reduction with sirolimus.
<b>Benzodiazepines</b>	
Midazolam Triazolam Diazepam	↑ $C_{max}$ (midazolam, triazolam) $t_{max}$ (triazolam) exposure and $t_{1/2}$ (midazolam, triazolam, diazepam) and bioavailability (midazolam). ↓ clearance (midazolam) via inhibition of hepatic and perhaps enteric CYP3A4.
<b>Corticosteroids</b>	
Methylprednisolone Dexametasone Prednisolone	↑ $C_{max}$ , $t_{max}$ (methylprednisolone) exposure (methylprednisolone, dexametasone, prednisolone) and $t_{1/2}$ (methylprednisolone, prednisolone) via inhibition of hepatic and enteric CYP3A4. Inhibition of P-gp may be involved (dexametasone).
<b>Calcium channel blockers</b>	
Felodipine	↑ $C_{min}$ , exposure and $t_{1/2}$ via inhibition of hepatic and enteric CYP3A4.
<b>Statins</b>	
Lovastatin Simvastatin Atorvastatin	↑ $C_{max}$ (lovastatin, simvastatin), exposure and $t_{1/2}$ (lovastatin, simvastatin, atorvastatin), via inhibition of hepatic and perhaps enteric CYP3A4. Use of these agents with ITRA is contraindicated. Alternative suitable agents include fluvastatin and pravastatin.
<b>Miscellaneous drugs</b>	
Antineoplastics Digoxin Quinidine Warfarin	Inhibition of CYP3A4 and/or P-gp may increase the exposure of certain antineoplastic agents resulting in significant toxicity. <b>See Table 7-13.</b>
<b>Drug affects ITRA</b>	
↓ ITRA exposure	Monitor antifungal response.
Phenytoin Rifampin/Rifabutin	↓ ITRA AUC and $C_{max}$ via induction of hepatic CYP3A4 by agent. <b>Use of these agents contraindicated with ITRA.</b>
Protease inhibitors	↑ ITRA exposure via induction of hepatic CYP3A4 by agent. ↓ PI dose.
See also Table 7-13. Key: $C_{max}$ , peak concentration; $C_{min}$ , trough concentration; CYP, cytochrome pathway; P-gp, P-glycoprotein; $t_{1/2}$ , half-life.	

### Echinocandins

The echinocandins produce few significant drug–drug interactions. Clinically relevant interactions with caspofungin may arise primarily from interactions with transport proteins. The most notable interaction with caspofungin involves rifampin. Caspofungin has no effect on the pharmacokinetics of rifampin but a transient increase in caspofungin plasma concentrations

occurs during the initial days of concomitant therapy with these two agents.<sup>211</sup> Caspofungin trough concentrations ultimately decline with continued co-administration and are also reduced when this agent is added to rifampin therapy. These findings suggest that rifampin reduces caspofungin distribution.<sup>211</sup> Rifampin is a known OATP1B1 substrate and inhibitor, and the inhibition of this protein could reduce the slow distribution



**Table 7-11** Clinically significant drug interactions with voriconazole (VORI)\*

Drug	Comments and recommended actions
<b>VORI affects drug: monitor for clinical toxicity and for toxic-range blood levels. Adjust dose</b>	
<b>Calcineurin inhibitors</b>	
Cyclosporine Tacrolimus Sirolimus	Significant ↑ $C_{min}$ of calcineurin inhibitors via inhibition of hepatic CYP3A4; sirolimus AUC and $C_{max}$ increased 5–10 fold. When starting VORI, reduce cyclosporine and tacrolimus dose by 50% and 75% respectively. Closely monitor calcineurin inhibitors blood levels and increase dose when discontinuing VORI. <b>VORI contraindicated with sirolimus.</b>
<b>Benzodiazepines</b>	
Midazolam (PO, IV)	↑ midazolam $C_{max}$ (PO) exposure and $t_{1/2}$ (PO, IV), bioavailability (PO) ↓ CL (PO, IV) via inhibition of hepatic and perhaps enteric CYP3A4.
<b>Analgesics</b>	
Ibuprofen Alfentanil Methadone	↑ $C_{max}$ (S-(+)-ibuprofen, (R)-methadone, (S)-methadone), exposure (S-(+)-ibuprofen, alfentanil, (R)-methadone, (S)-methadone), ↑ $t_{1/2}$ (S-(+)-ibuprofen, alfentanil). ↓ CL (alfentanil).
<b>Miscellaneous drugs</b>	
Antineoplastic agents Glipizide Warfarin Phenytoin Prednisolone Rifabutin	Inhibition of CYP 3A4, 2C19 or 2C9 may increase the exposure of certain antineoplastic agents metabolized by these enzymes resulting in serious toxicities. <b>See Table 7-13.</b> ↑ pharmacodynamic effect (warfarin) via inhibition of hepatic CYP2C9. ↑ $C_{max}$ (phenytoin, rifabutin) exposure (phenytoin, prednisolone, rifabutin) via inhibition of hepatic CYP2C9 (phenytoin) and CYP3A4 (prednisolone). The interaction with phenytoin and rifabutin is bidirectional.
<b>Drug affects VORI</b>	
↓ VORI exposure Phenytoin Rifampin/Rifabutin Carbamazepine Barbiturates	<b>Monitor antifungal response.</b> ↓ VORI AUC and $C_{max}$ via induction of hepatic CYP3A4 by agent. <b>Use of these agents contraindicated with VORI.</b> Only long-acting barbiturates affect VORI. May use phenytoin but need to increase VORI dose by ~100%.
↑ VORI exposure	<b>Monitor antifungal response and/or toxicity.</b>
Protease inhibitors	↑ VORI exposure via induction of hepatic CYP3A4 by agent.
↑ and ↓ VORI NNRTIs**	↑ and ↓ VORI exposure via inhibition or induction of CYP3A4 by agent. Monitor for VORI toxicity and lack of response to VORI.
See also Table 7-13. Key: $C_{max}$ , peak concentration; $C_{min}$ , trough concentration; AUC, area under the curve; CL, clearance; CYP, cytochrome pathway; $t_{1/2}$ , half-life. *Frequently monitor for adverse events and toxicity and reduce dose or discontinue drug in presence of symptoms. **NNRTIs: non-nucleoside reverse transcriptase inhibitors.	

of caspofungin and lead to increases in concentrations and exposure to this agent.<sup>183</sup>

There are few published data describing drug interactions with micafungin. Micafungin does not appreciably interact consistently with either cyclosporine or tacrolimus.<sup>212,213</sup> Similarly there are few published data from properly controlled studies investigating drug interactions involving anidulafungin. However, other studies have shown no significant interaction between anidulafungin, cyclosporine, tacrolimus and voriconazole.<sup>214-216</sup>

### Terbinafine

Terbinafine produces few significant drug–drug interactions. Clinically relevant interactions with voriconazole result primarily from interactions with CYP2D6.<sup>189</sup> Drugs that can produce clinically significant interactions with terbinafine are CYP2D6 substrates including psychopharmacologic agents (i.e., thioridazine, desipramine, nortriptyline, paroxetine, venlafaxine, codeine and dextromethorphan) and cardiovascular drugs (metoprolol, encainide, flecainide, propafenone and mexilitine).<sup>217</sup>

**Table 7-12** Clinically significant drug interactions with posaconazole (POSA)\*

Drug	Comments and recommended actions
<b>POSA affects drug: monitor for clinical toxicity and for toxic-range blood levels. Adjust dose</b>	
<b>Calcineurin inhibitors</b>	
Cyclosporine Tacrolimus	↑ $C_{min}$ of calcineurin inhibitors (cyclosporine, tacrolimus) via inhibition of hepatic CYP3A4 by POSA; tacrolimus AUC is increased by ~5-fold and its CL decreased by ~5-fold. Limited information on sirolimus. When starting POSA, reduce cyclosporine and tacrolimus dose by 25% and 50% respectively. Closely monitor calcineurin inhibitors blood levels and increase dose when discontinuing POSA.
<b>Benzodiazepines</b>	
Midazolam	↑ AUC (midazolam) ≈82% via inhibition of hepatic CYP3A4 by POSA.
<b>Miscellaneous drugs</b>	
Antineoplastics Glipizide Rifabutin	As with other azoles, inhibition of CYP 3A4 may increase the exposure of certain antineoplastic agents. Information on POSA and antineoplastic agents is limited. ↓ Plasma glucose via inhibition of hepatic CYP3A4 by POSA. Monitor blood glucose. ↑ Rifabutin $C_{max}$ and AUC via inhibition of hepatic CYP3A4 by POSA.
<b>Drug affects POSA</b>	
Cimetidine Phenytoin Rifabutin	↓ POSA exposure. <b>Monitor antifungal response. May need to ↑ POSA dose.</b> ↓ POSA AUC, $C_{max}$ via induction of hepatic CYP3A4 by cimetidine. ↑ <b>POSA dose when using cimetidine.</b> ↑ POSA CL by 90% via induction of hepatic CYP3A4 by phenytoin. ↓ POSA AUC and $C_{max}$ via induction of hepatic CYP3A4 by rifabutin. <b>Avoid use of POSA with phenytoin and rifabutin.</b>
NB: Experience with POSA is relatively limited and additional drug–drug interactions are likely. See also Table 7-13. Key: $C_{max}$ , peak concentration; $C_{min}$ , trough concentration; AUC, area under the curve; CL, clearance; CYP, cytochrome pathway. *Frequently monitor for adverse events and toxicity and reduce dose or discontinue drug in presence of symptoms.	

## Clinically significant drug–drug interactions that impact antifungal agents

### Azoles

Few drugs affect the pharmacokinetics of the azoles. Those that do are potent inducers of CYP enzymes including rifampin, rifabutin, phenytoin and phenobarbital. Often induction of azole metabolism by these agents leads to undetectable or significantly reduced serum concentrations of the affected azole that could compromise therapy.<sup>218</sup> Typically due to the magnitude of induction and the dose-dependent pharmacokinetics of the affected azole (with the exception of fluconazole), the interaction cannot be circumvented by increasing the dose of the azole and monitoring its serum concentrations.<sup>218</sup> These combinations should be avoided if possible but if alternative antifungal therapy cannot be used, then the serum concentrations of the affected azole should be closely monitored and the patient's clinical condition should be used as a guide in assessing the adequacy of the achieved concentrations.<sup>218</sup>

## Resistance to antifungal agents

The current era of new antifungal agents and the wider use of antifungal drug susceptibility testing has created a growing awareness of antifungal drug resistance. There are basically two clinical types of resistance: innate and acquired. Some species of fungi are inherently resistant to a particular antifungal agent whereas others have developed resistance over time, sometimes during therapy. Of particular concern recently has been the emergence of yeast isolates resistant to azole antifungal agents, especially fluconazole. In addition, there remains the unresolved issue of how in vitro resistance correlates with clinical resistance, which may vary depending on the fungal organism and antifungal agent being tested.

### Polyene resistance

Resistance to amphotericin B remains uncommon during treatment but reports of isolates exhibiting elevated MIC have become more common.<sup>59</sup> Resistance to polyenes is believed

**Table 7-13** Drug-drug interactions involving azole antifungal agents.

Cardiovascular agents				Psychotropic agents				
Azole Antifungal	Antiarrhythmics	Calcium-Channel Blockers	HMG Co-A Reductase Inhibitors "Statins"	Benzodiazepine Hypnosedatives/Anxiolytics	Non-Benzodiazepine Hypnosedatives/Anxiolytics	Anti-depressants/ Anti-psychotics	Antiepileptics	Gastric Acid Modifiers
Itraconazole AVOID	Digoxin Quinidine	Felodipine	Atorvastatin Lovastatin Simvastatin	Alprazolam Midazolam Triazolam	Buspirone	Haloperidol Pimozide Risperidone	Phenytoin	Antacids H <sub>2</sub> -Receptor Antagonists Omeprazole
USE CAUTION	Amiodarone	Amlodipine Isradipine Nifedipine Verapamil				Chlomipramine Nefazadone Trazodone	Carbamazepime Phenobarbital	Esomeprazole Lansoprazole Pantoprazole Rabeprazole
OK	Atenolol Lidocaine Sotalol		Fluvastatin Pravastatin Rosuvastatin	Bromazepam Diazepam Estazolam Oxazepam Temazepam	Zolpidem	Clozapine		
Fluconazole AVOID				Midazolam Triazolam				Omeprazole
USE CAUTION		Nifedipine	Atorvastatin Lovastatin Simvastatin	Diazepam	Buspirone	Amitryptiline Chlomipramine Fluoxetine	Phenytoin	
OK	Sotalol							
Voriconazole AVOID	Quinidine			Midazolam St. John's Wort		Pimozide	Carbamazepime Phenobarbital	
USE CAUTION		Felodipine	Lovastatin	Alprazolam Triazolam	Zolpidem		Phenytoin	Omeprazole
OK	Digoxin							Cimetidine Ranitidine
Posaconazole AVOID							Phenytoin	
USE CAUTION								
OK								



**Table 7-13** Drug–drug interactions involving azole antifungal agents.—cont'd

Antiretroviral agents									
Azole Antifungal	Steroids, Immunosuppressants	Analgesics/ Anesthetics	Antineoplastics	Protease Inhibitors	NRTIs	NNRTIs	Antibacterials/ Antimycotics	Oral Hypo-glycemics	Others
<b>Itraconazole</b> AVOID	Budesonide Methylprednisolone Prednisolone		Busulfan Vincristine Vinblastine Cyclophosphamide			Nevirapine	Sulfamethoxazole Clarithromycin Erythromycin Isoniazid Rifabutin Rifampin		EES Fexofenadine Levomethadyl
USE CAUTION	Dexamethasone Sirolimus Tacrolimus Cyclosporine	Alfentanil Methadone	Etoposide Docetaxel Paclitaxel Vinblastine	Indinavir Ritonavir Saquinavir				Nateglinide Pioglitazone Repaglinide Sulfonylureas	Sucralfate Oxybutrin Sildenafil Warfarin
OK	Mycophenolate Mofetil	Fentanyl Sufentanil	Gefitinib		Didanosine				Mestranol Selegiline Loperamide
<b>Fluconazole</b> AVOID	Cyclosporine	Alfentanil Celecoxib	Cyclophosphamide Paclitaxel				Rifabutin Rifampin	Glipizide Glyburide	Mestranol Warfarin
USE CAUTION	Sirolimus Tacrolimus	Ibuprofen	Docetaxel	Nelfinavir	Zidovudine		Clarithromycin	Nateglinide Sulfonylureas	Sildenafil
OK	Mycophenolate Mofetil			Indinavir Saquinavir	Didanosine	Delavirdine			Losartan Dapsone
<b>Voriconazole</b> AVOID	Sirolimus Tacrolimus	Alfentanil Methadone			Efavirenz		Rifabutin	Sulfonylureas	Warfarin Ergot alkaloids
USE CAUTION	Cyclosporine	Ibuprofen	Vincristine Vinblastine	Ampranavir Nelfinavir Ritonavir Saquinavir		Delavirdine Nevirapine	Rifampin		Certain BCPs
OK	Mycophenolate Mofetil Prednisolone			Indinavir			Azithromycin Erythromycin		
<b>Posaconazole</b> AVOID	Tacrolimus		Vincristine				Rifabutin		
USE CAUTION	Sirolimus Cyclosporine								
OK				Indinavir	Lamivudine Zidovudine			Glipizide	

**NB: Experience with posaconazole is relatively limited and additional drug–drug interactions are likely.** See also Tables 7-9 to 7-12. Avoid, interaction confirmed by controlled study, generally considered clinically significant, avoid combination if possible, or monitor closely; Use Caution, interaction noted in case report or can theoretically occur, generally not considered clinically significant, use combination cautiously; OK, combination studied and no or minimal interaction observed, not clinically significant, combination can be used; HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A; H2, histamine-2; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors; EES, ethynil estradiol.

to result from the production of a cell membrane possessing altered sterol content or composition.<sup>19,219-221</sup> These alterations can involve cell membranes with unusual sterols, altered ergosterol stereochemistry, and quantitative defects of ergosterol, which result in decreased polyene binding.<sup>222</sup>

Among *Candida* spp., in particular *C. lusitanae*, polyene resistance is usually due to a defect in ergosterol biosynthesis, most likely resulting from a mutation in the *ERG3* gene that produces altered  $\Delta 5,6$ -sterol desaturase activity.<sup>223,224</sup> This alteration leads to the production of abnormal sterols and decreased ergosterol production.<sup>223</sup> In addition to *ERG3*, mutations in *ERG11* (the gene that produces lanosterol 14 $\alpha$ -demethylase) and *ERG6* (a gene that is required for normal membrane function but is not essential for sterol biosynthesis) may also produce polyene resistance. *C. glabrata* isolates may also possess mutations in *ERG6* that lead to decreased membrane ergosterol and increased sterol intermediates, which may produce reduced polyene susceptibility.<sup>225</sup> In other *Candida* spp., including *C. krusei* and *C. tropicalis*, resistance is associated with diminished membrane sterols.<sup>226</sup> Additional polyene resistance mechanisms in *Candida* spp. may also involve oxidative changes in the fungal cell that may result from diminished aerobic respiration.<sup>227</sup>

## Flucytosine resistance

As discussed above, flucytosine has a relatively narrow spectrum of activity, mainly against *Candida* spp. and *C. neoformans*.<sup>30</sup> Monotherapy with this agent rapidly leads to clinical resistance. Several mechanisms of resistance are possible given the multiple intracellular enzymatic steps required for its action. These include alterations in the target enzymes uridine monophosphate pyrophosphorylase (most common), cytosine permease, and cytosine deaminase, or increased production of pyrimidines.<sup>30</sup> In addition, *C. albicans* serotypes A and B seem to have different sensitivities, with serotype A being susceptible and serotype B generally resistant to flucytosine.<sup>30,228</sup> Prevalence of resistance in yeast isolates varies widely with geographic location from 5% to 43%.<sup>228,229</sup>

## Azole resistance

Development of the azole group of antifungal compounds vastly improved the treatment of fungal infections and has led to their widespread use. Consequently, with extensive use have come reports of resistance to these agents, particularly fluconazole.<sup>56,230-237</sup> Several excellent reviews on the subject have been written.<sup>219,221,237-241</sup>

Azole resistance in *Candida* has been the most widely observed and studied for fluconazole and *C. albicans*; however, azole resistance to other azoles among other *Candida* spp. has been reported and studied. Resistance to the azoles can result from quantitative or qualitative modifications of target enzymes, reduced access of the drug to the target enzyme or by a combination of these mechanisms. Qualitative modifications in target enzymes result from point mutations in *ERG11*, the gene responsible for producing 14 $\alpha$ -demethylase, which is the principal target of the azoles. In certain *Candida* spp. these mutations produce differential susceptibilities across the azole class. As discussed above, this may be due to the differing affinities azoles have for secondary targets in the ergosterol

biosynthesis pathway. Alternatively, the different chemical structures of the azoles may also contribute to this differential activity. Unlike fluconazole and voriconazole, itraconazole and posaconazole possess large hydrophobic side chains. The point mutations may impact itraconazole and posaconazole less than fluconazole or voriconazole because these side chains may afford additional contacts with the target enzymes.<sup>242</sup> Quantitative modifications in target enzymes also result from mutations in *ERG11*. Overexpression of the gene results in overproduction of the target enzymes, which then necessitates higher intracellular azole concentrations to inhibit all the target enzyme.<sup>224,243</sup>

The other primary mechanism by which fungi resist the effects of the azole antifungal agents involves the active efflux of the azole out of the cell via the activation of two types of efflux transport proteins encoded by either MDR or CDR genes.<sup>19,224,235,243,244</sup> In general, upregulation of MDR genes primarily affects fluconazole, whereas upregulation of the CDR genes confers resistance to multiple azoles.<sup>224,243,245-249</sup> The two primary mechanisms of resistance (qualitative/quantitative changes in target enzymes and active efflux) may also act sequentially or in concert to produce resistance to the azoles.

## Echinocandin resistance

As a class, the echinocandins are the most recent addition to the antifungal arsenal. Consequently, to date their use has been too limited to assess whether significant resistance will impact this class. Clinical reports of echinocandin-resistant *Candida* spp. are rare and to date such reports for *Aspergillus* spp. are lacking. Challenges in terms of developing standard in vitro susceptibility tests and the lack of significant numbers of resistance isolates to this class have hindered the establishment of widely accepted susceptibility breakpoints for this class. Therefore, compared to other classes, the understanding of resistance mechanisms to this class is still evolving.

Isolates can demonstrate either intrinsic or acquired resistance to the echinocandins. Organisms that demonstrate intrinsic resistance possess either insufficient target enzyme  $\beta 1,3$ -D-glucan synthase or a mutated form of the enzyme that precludes echinocandin binding.<sup>57,250-252</sup> Given the narrow spectrum of this class, many organisms demonstrate intrinsic resistance, including but not limited to *Cryptococcus*, *Trichosporon* spp., *Fusarium* spp., and the agents of zygomycosis.<sup>253</sup> Acquired resistance to the echinocandins involves the mutation of the *FKS1* gene, which encodes a subunit of  $\beta 1,3$ -D-glucan synthase.<sup>253</sup> Although most of the work done to date in this area has been with caspofungin, data suggest that the mutations in this gene may confer reduced echinocandin susceptibility in a wide array of fungal species.<sup>253</sup>

## Terbinafine resistance

Terbinafine resistance appears to be a rare occurrence in dermatophytes and *C. albicans*.<sup>189</sup> Data suggest that some pathogenic fungi appear to have the potential to develop resistance to terbinafine and, like the azoles, resistance to terbinafine may be mediated by an overexpression of multidrug efflux transporter, specifically the CDR genes.<sup>189</sup> Nonetheless, to date overexpression of CDR transport proteins has yet to develop into a clinically significant problem.

## Conclusion

The therapy of fungal infections, once limited and toxic, has undergone an explosive period of development in recent years. Since the middle of the 1990s, new agents with novel mechanisms of action, enhanced potency, improved pharmacokinetics, and substantially less toxicity have reached the marketplace. The lipid amphotericin B formulations extended the life of this valuable agent by reducing its notable toxicity. The azole group of compounds has provided excellent alternatives to amphotericin B in the treatment of most clinically important mycoses. Research into the development of newer antifungal agents that focused on novel targets yielded the echinocandins, which are the first new class of compounds in several decades. This class provides another alternative in the treatment of candidiasis and aspergillosis.

With all the choices, clinicians must be cognizant of differences in the spectrum of activity, pharmacokinetics and drug interaction potential of antifungal agents so that they may select the most appropriate drug for their patient. In addition, antifungal drug resistance is also becoming increasingly recognized as an important clinical problem. Azole resistance, especially to fluconazole, has been frequently reported. As more antifungal agents become available, drug resistance will likely continue to be a problem. Further advances in antifungal chemotherapy will be necessary to improve management of invasive mycoses in the future.

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# Candida

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## Introduction

Several *Candida* spp., most notably *C. albicans*, are ubiquitous human commensals. They become pathogens in situations where the host's resistance to infection is lowered locally or systemically. In such circumstances, *Candida* spp. can cause disease in virtually every location of the human body, resulting in superficial, locally invasive or disseminated infection. The prevalence of *Candida* infections in medical practice is high, and changes in the way in which patients can become susceptible to *Candida* have led to changes in the epidemiology and clinical presentation of these infections, and to a steady rise in their incidence over the last 45 years.

Candidiasis refers to infection caused by any of the >160 species of the genus *Candida*. Currently, *Candida* spp. are the forth most common cause of nosocomial bloodstream infections (BSI) in the US<sup>1,2</sup> and have been associated with significant mortality.<sup>3</sup> *Candida* is also the leading cause of invasive fungal infection in critically ill adult and neonatal patients cared for in intensive care units (ICU), and the second cause of invasive fungal infection in severely immunocompromised patients such as those with cancer and recipients of hematopoietic stem cell and bone marrow (HSCT/BMT) or solid organ transplantation (SOT).

In this chapter, we will present an overview of the microbiology of *Candida* and the infectious syndromes caused by this fungus. For more information on specific aspects of various candidal infections, please refer to other chapters of the textbook.

## Candida species: the pathogen

Several *Candida* species have caused infection in humans (Table 8-1) while many others have been occasionally described as pathogens,<sup>4</sup> although the evidence for the clinical involvement of the less common species is not always of the most convincing quality.

## Taxonomy and mycology

A detailed review of *Candida* taxonomy was published in 1998.<sup>5</sup> Currently, *Candida* belongs to the Kingdom: Fungi, Phylum: Ascomycota, Subphylum: Ascomycotina, Class:

Ascomycetes, Order: Saccharomycetales, Family: Saccharomycetaceae, Genus: *Candida* ([www.doctorfungus.org/thefungi/candida\\_spp.htm](http://www.doctorfungus.org/thefungi/candida_spp.htm)).

## Cell biology and enzymology

Because of its predominant medical importance, *C. albicans* has been investigated more extensively than other *Candida* species. Its growth characteristics, metabolic processes and enzymologic characteristics are all similar to those of eukaryotes and particularly similar to *Saccharomyces cerevisiae*. Two major differences between these species are the existence of a sexual cycle in *S. cerevisiae* (*C. albicans* has no equivalent) and a greater tendency to undergo morphologic variation in response to changes in microenvironment in *C. albicans*.

Blastoconidia of *Candida* species multiply by means of a typical yeast budding process. *C. albicans* can also develop as true fungal hyphae.

Polysaccharides in *Candida* species are primarily in the cell walls. The main components of the *Candida* cell wall are phosphorylated mannans, glucans and a smaller amount of chitin. Polypeptides and proteins are intimately bound with cell wall polysaccharides and the fine structures of the various wall phospho-glycopeptide oligomers and polymers account for differences in antigenic structures, gross hydrophobic properties and specific adhesions to host cells and other surfaces between *Candida* species and strains. The expression of cell wall macromolecules can also vary from cell to cell and even within different portions of the wall in the same cell.

Indeed, the cell wall of *C. albicans* is now recognized as a dynamic, constantly changing structure that contains enzymically active proteins such as enolase and *N*-acetyl glucosaminidase, ubiquitin-like epitopes and a protein related to the hsp70 (heat shock protein) family. The variable expression of cell wall proteins is presumably the result of a complex and dynamic system of regulation. The wall contains three types of adhesin molecule.<sup>6</sup> One is a glycoprotein. It is expressed specifically on the surface of *C. albicans* hyphae mimicking human complement components C3d and iC3b. A second adhesin type is the protein moiety of a glycoprotein that binds to host glycosides containing fucose or *N*-acetyl glucosamine. The third type involves the polysaccharide portion of a manoprotein that binds to unknown host receptors.

The structure of mannan polysaccharides in the wall of *C. albicans* has been extensively studied.<sup>7,8</sup> The nature of the mannan structure in the walls can be altered by changes in the external pH and temperature. Glucan polymers in the *C. albicans* cell wall are located deeper in the wall than mannan.

Lipids in *C. albicans* are predominantly phospholipids and sterols, with ergosterol the principal membrane sterol. These lipids provide the site of action for the synthesis of enzyme(s) involved in cell wall morphogenesis and antifungal action. Lipid alterations can occur during a yeast-to-mycelium transition. For more details readers are referred to a review on this topic.<sup>9</sup>

*C. albicans* can grow over a very wide pH range, from below 2.0 to almost 8.0, and under microaerophilic and even anaerobic conditions as well as the more normal aerobic atmospheres of incubation. Glucose, galactose and sucrose are all substrates for growth of the fungus, and nitrogen requirements can be met by relatively low concentrations of ammonium ions. Carbohydrate catabolism in *Candida* spp. occurs via the glycolytic pathway and the tricarboxylic acid cycle. In addition to the conventional eukaryotic pathway of oxidative phosphorylation several *Candida* spp., including *C. albicans*, have an inducible, cyanide-resistant alternative respiratory pathway.

Many enzymes in *C. albicans* have been characterized.<sup>10</sup> One of the most extensively studied groups of enzymes are the secreted aspartyl proteinases (Sap) produced by *C. albicans*, *C. dubliniensis*,<sup>11</sup> *C. guilliermondii*,<sup>12</sup> *C. parapsilosis*,<sup>13</sup> and *C. tropicalis*.<sup>14</sup> Distinct isoforms of Sap of *C. albicans* are important virulence factors for different types of candidiasis. These enzymes produce non-specific proteolysis of host proteins involved in defenses against infection. Their different profiles of pH-dependent irreversible denaturation may partially explain differences in virulence of *Candida* species.<sup>15</sup>

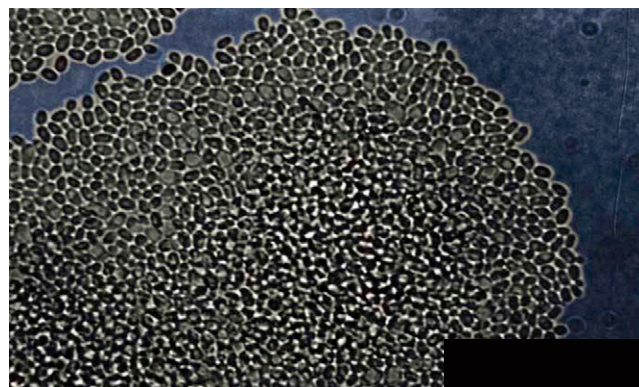
The Sap superfamily in *C. albicans* includes at least 10 isoenzymes (the genes are referred to as SAP1 through SAP10).<sup>12,16-19</sup> Different Saps are associated with different location within the yeasts and different pathogenesis. Predominant expression of Sap1-3 has been shown to be crucial for superficial infections in experimental mucosal and cutaneous candidosis, whereas Sap 4-6 might be important for systemic disease.<sup>20,21</sup> The SAP gene family of *C. tropicalis* and *C. dubliniensis* are likely to contain four<sup>14</sup> and seven members,<sup>11</sup> respectively.

*C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis*, and *C. tropicalis*<sup>22-24</sup> also

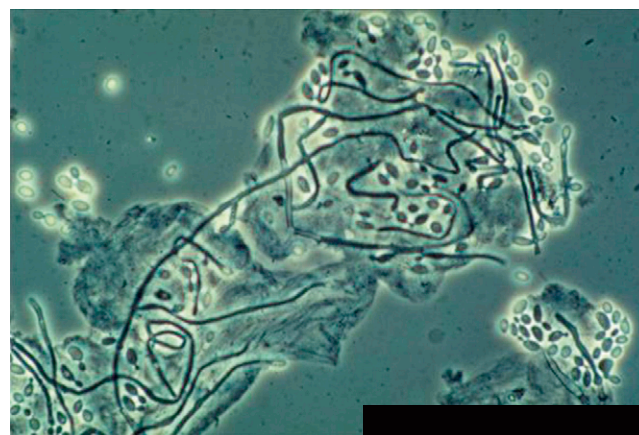
produce phospholipases. Such enzymes are important for growth control of the yeast, remodeling of the fungal cell membrane and invasion through hydrolysis of phospholipids of the host tissues.<sup>25-27</sup> Phospholipase B is essential for *C. albicans* virulence, and it is secreted by the yeast during the infection process.<sup>24</sup>

## Morphogenesis ("Dimorphism")

*C. glabrata* grows as a small, elliptical, unicellular budding yeast (Fig. 8-1). This growth contrasts with that of *C. albicans*, *C. krusei*, *C. parapsilosis* and *C. tropicalis* which form larger elliptical budding yeasts and can also form pseudohyphae which are well-developed multicellular filaments (Fig. 8-2). *C. krusei* and *C. parapsilosis* can be considered dimorphic because they exhibit budding and pseudohyphal forms. In addition to buds and pseudohyphae, *C. albicans* and *C. tropicalis* can form true hyphae (Fig. 8-3). The presence of budding yeasts and pseudohyphae in infected tissue is usually indicative of candidiasis although similar findings may be observed with certain opportunistic mould infections (e.g., *Paecilomyces lilacinus*).



**Figure 8-1** *Candida glabrata*. Microscopic morphology. Growth on corn agar. Round to oval blastoconidia. Hyphae and pseudohyphae are absent. (Courtesy of Deanna A. Sutton. Copyright © 2003 Doctorfungus Corporation.)



**Figure 8-2** Yeast cells and pseudohyphae of *C. albicans* in material from the oral cavity, KOH preparation, phase-contrast microscopy. (Courtesy of M. McGinnis. Copyright © 2000 Doctorfungus Corporation.)

**Table 8-1** List of *Candida* spp. that are opportunistic human pathogens

### Species commonly implicated in human infections

*C. albicans*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis* and *C. tropicalis*

### Species uncommonly implicated in human infections

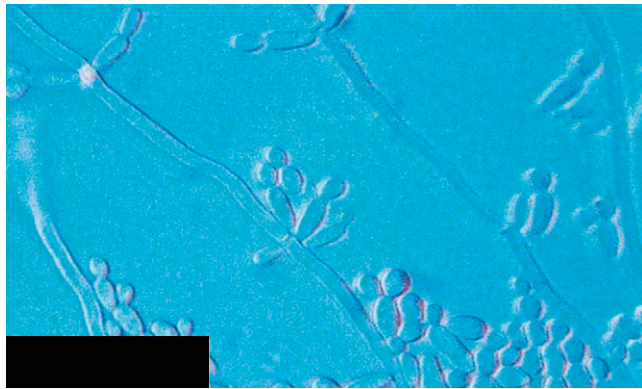
*C. albidus*, *C. catenulate*, *C. chiropterorum*, *C. ciferrii*, *C. dubliniensis*, *C. famata*, *C. haemulonii*, *C. humicola*, *C. inconspicua*, *C. kefyr*, *C. lambica*, *C. lipolytica*, *C. norvegensis*, *C. pelliculosa*, *C. pintolopesii*, *C. pulcherrima*, *C. rugosa*, *C. utilis* and *C. zeylanoides*

Finally, under suitable incubation conditions, *C. albicans* can also form large, refractile chlamydoconidia (Fig. 8-4). Several genes are thought to be involved in regulating the morphogenetic processes.<sup>28</sup>

The clinical significance of dimorphism in *C. albicans* has sometimes been overstated. It is untrue that only hyphal forms are invasive while yeast forms are associated with the commensal state: both forms can penetrate host tissues and both forms express potential virulence attributes.<sup>29</sup> Indeed, both forms, budding and filamentous, likely play a role in the progression of infection in humans. The ongoing formation and detachment of unicellular buds can facilitate hematogenous dissemination of the yeast following angioinvasion. In contrast, filamentation presumably enhances the ability of *Candida* species to invade tissues.<sup>30-32</sup>

The signals that trigger the growth of the various forms (budding yeasts, pseudohyphae and hyphae) include pH of the microenvironment, carbon and nitrogen availability, oxygenation, serum, hormones, and the density of the *Candida* cells within the infected host.

pH-sensitive genes involved in growth and morphology have been identified indicating that the importance of these genes might vary according to the site of infection. Similarly,



**Figure 8-3** Blastoconidia and true hyphae of *C. albicans*. (Courtesy of Deanna A. Sutton. Copyright © 2003 Doctorfungus Corporation.)



**Figure 8-4** Thick-walled chlamydoconidia of *C. albicans* are formed. 400×, 25°C. Corn meal agar plate inoculated by the Dalmau technique. (Courtesy of M. McGinnis. Copyright © 2000 Doctorfungus Corporation).

two pH-sensitive genes involved in cell wall synthesis have been identified and expression of both genes is required for normal growth of budding and filamentous forms.<sup>33-35</sup>

Genes which regulate the switch from the budding yeast to the filamentous form have been described.<sup>36,37</sup>

Also regulating filamentous growth are various protein kinases in the Cdc2 subfamily and quorum sensing.<sup>38,39</sup>

## Phenotypic switching in *C. albicans*

Colonies of *C. albicans* on agar media sometimes show variations in form, particularly after long periods of incubation. This characteristic has been defined as expression of a phenomenon called phenotypic switching. Changes from one colony form to another often occur at a very high frequency ( $10^{-2}$ ) in sequential subcultures from a single clone, with new colony variants and revertants to the original form appearing almost at random. The frequency of the switching phenomenon is too high to result from gene mutation, and too low to be attributable to mass conversion, in which all cells in a population change their phenotype in response to environmental changes. It is likely that the switching phenomenon serves as a type of master system in *C. albicans* for rapid responses at the level of individual cells to changes in the local microenvironment. Such responses would explain differences described between epitopes expressed at the surfaces of individual cells, and might be linked to the changes in cell morphology sometimes seen within individual cell units. It has been postulated that phenotypic switching explains the ability of *C. albicans* to survive in many different environmental microniches within a mammalian host.<sup>40</sup>

*C. albicans* uses a common set of conserved pathways to regulate dimorphism, mating and phenotypic switching. All these pathways regulate the expression of hyphae-specific and/or phase-specific genes which encode proteins that contribute directly or indirectly to pathogenesis and virulence of *C. albicans*. Therefore, virulence genes are co-regulated with cell morphogenesis.<sup>28</sup>

## Molecular biology of *C. albicans*

Much progress has been made in knowledge of the molecular biology of *C. albicans*. A scan of the Internet website at the University of Minnesota (<http://alces.med.umn.edu>) will give the reader access to current details of genes from *C. albicans* that have been cloned: hundreds of DNA sequences have been registered in the official Genbank database, and hundreds more can be found listed at the website described above. A description of all known molecular biologic findings in pathogenic *Candida* species is beyond the scope of this chapter.

## Epidemiology and pathogenesis

The *Candida* spp. known as human pathogens reside primarily in the gastrointestinal tract (gut) but are also commensals in the vagina and urethra, on the skin and under fingernails. *C. albicans*, the species most often associated with human disease, is also recovered from various sources apart from vertebrates, including the atmosphere, fresh water, sea water and soil. It is an occasional contaminant of foodstuffs and can be



recovered from fomites, particularly items that have contacted humans directly, such as clothing, bedding and toothbrushes. Places where *C. albicans* is found away from animals are almost invariably the result of human and animal contamination rather than primary habitats.<sup>41-43</sup>

Estimates of the prevalence of *Candida* spp. as human commensals vary considerably according to site and population sampled and sampling method. The prevalence of oral *Candida* colonization is around 6% (2–37%) among healthy subjects and 47% (13–76%) among hospitalized patients.<sup>44</sup> Oral carriage rates may be higher in certain settings such as in HIV-infected patients with low CD4 counts,<sup>45</sup> denture users with denture stomatitis,<sup>46</sup> diabetic patients,<sup>47</sup> patients receiving antineoplastic chemotherapy<sup>48,49</sup> and children.<sup>50</sup> Among diabetics, local factors such as smoking and presence of dentures additionally promote oral colonization.<sup>47</sup>

It seems likely that virtually 100% of humans may carry one or more *Candida* species in the gut (from the duodenum to the colon), and that the numbers of yeasts carried at any point in the gut often increase to levels that become detectable in the mouth and feces in illness or other circumstances where the host's microbial suppression mechanisms become reduced.

Dry, glabrous skin is rarely colonized by *Candida* species and the species that predominate in skin samples are *C. guilliermondii* and *C. parapsilosis*, rather than *C. albicans*. Furthermore, the half-life of *Candida* spp. on the skin of hands is only a few minutes; however, the capability of transmission of the yeasts from hand to hand and to inanimate objects was confirmed,<sup>51</sup> and extended survival of the yeasts on inanimate objects in these experiments suggests that *Candida* spp. may be transmitted to inanimate sources from humans and animals.

## Virulence factors

The state of the host is of primary importance in determining *Candida* pathogenicity. In fact, *Candida* spp. are considered opportunistic pathogens and for disease to occur there must be a breakdown in host defenses. A comprehensive model has been described that shows how *C. albicans* strains that reach the bloodstream through gut translocation or central venous catheters (CVC) interact with host defenses and exit the intravascular compartment to invade deep tissues.<sup>52</sup> However, there are factors associated with the organism that contribute to its ability to cause disease and explain the difference in the pathogenicity among species.

Numerous virulence factors exist and may play different roles with differing sites and stages of a given infection. Known virulence factors include ambient pH,<sup>33,34</sup> calcineurin (a calcium-regulated signaling enzyme),<sup>53</sup> and multiple adherence mechanisms that allow initial attachment to host tissue (or plastic foreign bodies) and subsequent proliferation. These include a hypha-specific surface protein, Hwp1, needed to form stable attachments to epithelial cells,<sup>54</sup> mutations in genes that regulate the switch from budding yeast to a filamentous form,<sup>55</sup> a gene (INT1) coding for a *C. albicans* surface protein<sup>56</sup> and mannosyl transferase.<sup>57</sup>

Adherence of *Candida* spp. to a wide range of tissue types and inanimate surfaces is essential in the early stages of colonization and tissue invasion. *C. albicans* adheres more strongly to epithelial cells than *C. tropicalis*, followed by *C. parapsilosis*. These findings are in agreement with the virulence ranking

of these species.<sup>58</sup> This adherence is achieved by a combination of specific (ligand–receptor interaction) and non-specific (electrostatic charge, van der Waals forces) mechanisms.<sup>59</sup> Germinated *C. albicans* cells adhere to host tissue more readily than do yeast phase.<sup>60</sup>

The hydrophobicity of the cell surface of *C. albicans* plays an important role in the adhesion of the organism to eukaryotic cells and inert surfaces. The glycosylation of the cell surface mannoproteins may affect this hydrophobicity, therefore affecting the adhesion of *C. albicans* to epithelial cells.<sup>61</sup> Blastospores of *C. albicans* are hydrophilic, but the germ tube formation is associated with a significant rise in cell surface hydrophobicity.<sup>62</sup>

The mannans (glycoproteins present on the cell surface of *C. albicans*) also contribute to the virulence of *C. albicans* mainly by affecting the yeast cell surface hydrophobicity, leading to changes in adherence to host tissues, and also by suppressing immune response.<sup>63</sup> Mannoproteins released from *C. albicans* bind to human red blood cells and causes hemolysis. Hyphae of *C. albicans* bind to human hemoglobin, but not yeast cells. The amount of hemoglobin receptor is significantly higher in hyphal cells than on yeast cells. Only the hyphal cells of *C. albicans* use hemoglobin as a source of iron.<sup>64-66</sup>

Enzyme production by *Candida* spp. is an important virulence factor. These enzymes include extracellular proteinases, phospholipases, lipases, hydrolytic enzymes and adhesions.<sup>67-72</sup>

The most extensively studied groups of enzymes are the secreted aspartyl proteinases (Sap) and the phospholipases (see section above on Cell Biology and Enzymology of *Candida* spp.).

The phenotypic switching phenomenon may be associated with the relative virulence of the species.<sup>73</sup> The rate of phenotypic switching is higher in strains of *C. albicans* from invasive infections than in those colonizing superficial sites.<sup>74</sup> Expression of cell wall glycoproteins, secretion of proteolytic enzymes, hyphae formation, susceptibility to killing by neutrophils and/or their oxidants,<sup>75</sup> and azole resistance are all contributors to the organism virulence and have all been associated to the switching phenomenon. Therefore, phenotypic switching contributes to the virulence of *C. albicans* by facilitating its ability to survive, invade tissues and escape host defenses.<sup>73</sup> On the other end, neutrophils themselves can augment the switching process towards more susceptible strains.<sup>75</sup>

Another potential virulence factor is the resistance to the thrombin-induced platelet microbicidal protein (tPMP).<sup>76</sup> Susceptibility or resistance of *Candida* spp. to tPMP has been shown to have an effect on the outcome of experimental endovascular infections. In all target tissues, the extent of candidal clearance by fluconazole is greater in animals infected with the tPMP-susceptible strain than in those infected with the tPMP-resistant strain.<sup>77</sup>

## Routes of transmission of *Candida* species

The predominant source of infection in all diseases caused by *Candida* spp. is the patient. The necessary requirement for invasive disease is a lowering of a host anti-*Candida* barrier. Transmission of *Candida* spp. from the gut to the bloodstream requires yeast overgrowth in the gut,<sup>78</sup> and is favored by loss of the integrity of the gut mucosa.<sup>79,80</sup> Thus, the endogenous commensal source accounts for the great majority of *Candida* spp. infections.

The importance of exogenous transmission of *Candida* depends greatly on the nature of the disease involved. Outbreaks of *Candida* spp. infection resulting from contaminated materials have been described including postsurgical endophthalmitis caused by contaminated intravitreal solutions,<sup>81</sup> candidemia resulting from contaminated total parenteral nutrition (TPN) solutions,<sup>82</sup> contaminated blood pressure transducers,<sup>83</sup> and contaminated suppositories.<sup>84</sup> Transmission of *Candida* species from staff to patient and from patient to patient has been demonstrated,<sup>85,86</sup> but such routes seem to be of significance only in specialized, relatively closed settings such as burn,<sup>87</sup> geriatric,<sup>88</sup> hematology,<sup>89,90</sup> ICU,<sup>91-93</sup> and transplantation units.<sup>94,95</sup>

One particular instance of *Candida* spp. transmission concerns neonates. Many newborns acquire a *Candida* flora from the maternal vagina at the time of birth or during gestation. Indeed, vulvovaginal candidiasis (VVC) occurs in up to 56% of pregnant women, especially in the last trimester.<sup>96</sup> However, non-perinatal nosocomial transmission of *Candida* spp. to neonates can also occur.<sup>97</sup> Several outbreaks of candidemia in neonatal ICU (NICU) have been identified<sup>98,99</sup> and the hands of the hospital personnel may be a potential reservoir for transmission,<sup>100</sup> particularly as *Candida* spp. carriage on hands of hospital personnel may be as high as 58%.<sup>101</sup>

Although most women with VVC are infected with an endogenous strain, sexual transmission between partners,<sup>102,103</sup> especially in the setting of receptive oral sex, has been suggested.<sup>104,105</sup> While it is likely that in most cases of VVC the infecting fungus travels from the anus to the introitus, transmission to this site from the mouth or hands is also possible. Most cases of recurrence of VVC have been ascribed to relapse with the same strain,<sup>106</sup> rather than to infection with a new strain. Uncommon intermediate reservoirs for vaginal reinfection include the urethra and the fingernails.

Among IV drug abusers (IVDA) with hematogenous candidiasis,<sup>107</sup> the infection may be transmitted through IV injection of heroin dissolved in contaminated lemon juice. The lemon juice originally becomes contaminated most probably with yeasts from the heroin users themselves, serving as an example of indirect transmission of an endogenous strain.<sup>108-110</sup>

## Clinical prevalence of different *Candida* species

*C. albicans* is the most commonly implicated *Candida* spp.<sup>111</sup> and infections of genital, cutaneous, and oral sites almost always involve this species. The most frequent non-*albicans* species regarded as pathogens are *C. dubliniensis*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. lusitanae*, *C. parapsilosis*, *C. pseudotropicalis*, *C. tropicalis*.

*C. albicans* is the predominant species causing oropharyngeal candidiasis (thrush) among HIV-positive patients.<sup>45,112,113</sup> The widespread use of fluconazole prophylaxis in HIV-infected patients has resulted in the appearance of fluconazole-resistant strains of *C. albicans*<sup>113-115</sup> and increasing frequency of non-*albicans Candida*, especially with late-stage AIDS.<sup>116</sup> However, since highly active antiretroviral therapy (HAART) became available, the rate of carriage of fluconazole-resistant *C. albicans* has greatly declined. A decline in carriage of fluconazole-susceptible *C. albicans* strains was not observed, suggesting that carriage of fluconazole-resistant *C. albicans*

strains is a function of the host's immune status.<sup>117</sup> Of note, *C. dubliniensis* may be misdiagnosed as a fluconazole-resistant *C. albicans*.<sup>118</sup>

*Candida* spp. are now the fourth most common organism isolated from blood of hospitalized patients in the US.<sup>2</sup> Data from a nationwide surveillance study (1995–2002) showed that among 1890 *Candida* isolates causing nosocomial BSI, *C. albicans* was the most common (54% of episodes), followed by *C. glabrata* (19%), and *C. parapsilosis* and *tropicalis* (11% each). During this 7-year period, the proportion of *C. albicans* and *parapsilosis* increased in contrast to decreasing proportions of *C. glabrata* and *tropicalis*. Species distribution was also studied in a worldwide surveillance program (1997–2003)<sup>111</sup> which included 134,715 consecutive clinical isolates of *Candida* from 127 medical centers in 39 countries. Again, *C. albicans* was the most common (66%), followed by *C. glabrata* (~11%), *C. tropicalis* and *C. parapsilosis* (~6% each) and *C. krusei* (~2%). Over time, a trend towards a decrease in *C. albicans* and an increase in *C. tropicalis* and *C. parapsilosis* was observed.

Changes in species distribution can occur not only over time but also in different locations. Although exposure to antifungal agents has long been considered the main factor for this change (e.g., exposure to fluconazole increased infections by *C. glabrata* and *C. krusei*,<sup>119,120</sup> recent data show that multiple factors can lead to changes in species distribution. Severe immunosuppression, prematurity, critical illness, exposure to broad-spectrum antibiotics, and older age may lead to a reduction in the rates of *C. albicans* in favor of the non-*albicans* spp., in particular *C. glabrata*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis*.<sup>111,121-128</sup> Use of IV catheters and lack of compliance with hand washing by healthcare workers were reported to increase *C. parapsilosis* infections.<sup>123,129</sup>

These factors may explain the differences in species distribution in various parts of the world. For example, Latin America has the lowest rate of *C. albicans* and *C. glabrata* infections while these species are the most commonly isolated in the US and Denmark.<sup>111</sup>

Site colonization by more than one *Candida* species is not uncommon. Studies conducted amongst healthy individuals,<sup>44</sup> patients with hematologic malignancies,<sup>44,130</sup> diabetes mellitus,<sup>131</sup> HIV infection,<sup>45</sup> nasopharyngeal cancer,<sup>132</sup> and geriatric patients<sup>88</sup> indicate that colonization by more than one *Candida* species may be as high as 44%. Individuals from whom only *C. albicans* can be isolated are usually colonized with a single strain type; in terms of population genetics, the colonization is described as clonal.<sup>133</sup>

However, colonization by more than one biotype of *C. albicans* (polyclonal colonization) ranges from 3% to 55% amongst healthy individuals,<sup>44</sup> patients with hematologic malignancies,<sup>44,130</sup> HIV infection,<sup>130</sup> and among geriatric patients.<sup>88</sup> Therefore, although most commensal *C. albicans* populations tend to be principally clonal, small variations of strain type are encountered in different anatomic niches. These arise by microevolution as a result of genetic rearrangements.<sup>134,135</sup> Simultaneous *Candida* colonization of more than one site may involve the same or different strains. Concurrent isolation of similar species or biotypes is the most common finding, especially when the sites are anatomically related; >90% of *Candida* strains isolated simultaneously from vagina, urethra and anus represent the same species or *C. albicans*

biotype while only 61–75% of simultaneously isolated anal and oral *Candida* strains are the same.<sup>130,136</sup>

## Disease spectrum

Despite the substantial overlap of characteristics of the various syndromes of invasive candidiasis, it is useful to divide these syndromes into those that involve the bloodstream (hematogenous candidiasis) and those that present as infection in a specific organ (deep organ candidiasis).

### Hematogenous candidiasis

Candidemia refers to the isolation of pathogenic species of *Candida* from a blood culture specimen. Such a simple definition does not, however, do justice to the broad spectrum of infections associated with candidemia. Indeed, in non-neutropenic hosts, candidemia may remain uncomplicated with a relatively benign course or become complicated with end-organ involvement such as osteomyelitis, endophthalmitis or invasion of other deep organs. Similarly, candidemia in neutropenic patients may remain limited to the bloodstream (uncomplicated) or evolve into life-threatening complications such as acute disseminated candidiasis, chronic disseminated candidiasis or single deep organ candidiasis.

### Deep organ candidiasis

Most deep organ candidal infections develop as a result of undetected hematogenous candidiasis; occasionally, infections may arise as a result of direct inoculation of the fungus in certain organs; an example is peritonitis which usually develops in patients with peritoneal dialysis catheters or following bowel injury.

Because of our limited ability to detect hematogenous and deep organ infection, drawing a sharp distinction between various forms of invasive candidiasis is difficult.

Table 8-2 summarizes the clinical forms of *Candida* infection and the settings in which they are encountered. The most common forms of disease caused by *Candida* species involve the gastrointestinal tract (typically the oral cavity), female genitalia and skin and nails.

## *Candida* infections of the gastrointestinal tract

Oral *Candida* infections (oral thrush) occur predominantly in patients with systemic or local immunosuppression or with exposure to other factors that favor candidal overgrowth and invasiveness. Immunosuppressed individuals at risk include newborns with birth asphyxia,<sup>137</sup> malnourished<sup>138</sup> or diabetic patients,<sup>139</sup> patients with HIV infections,<sup>140</sup> those receiving corticosteroid or cytotoxic chemotherapy<sup>141</sup> and those undergoing maxillofacial radiotherapy,<sup>142</sup> SOT or BMT.<sup>141</sup> Prolonged therapy with antibiotics<sup>139,143</sup> or inhaled corticosteroids<sup>144</sup> and denture wearing are additional risk factors.<sup>143</sup>

The prevalence of oral thrush in AIDS patients approaches 100%, particularly when CD4 counts are <200/μL, and among smokers.<sup>145,146</sup> However, the introduction of HAART has resulted in a significant decrease in the incidence of oral thrush to as low as 2–4%.<sup>117,147</sup> Among cancer patients undergoing antineoplastic therapy, the incidence of oral thrush ranges between 28% and 38%.<sup>148,149</sup>

Esophageal candidiasis (EC) remains one of the most common AIDS-defining illnesses in the era of HAART. A pan-European longitudinal, prospective observational study (EuroSIDA) reported that 15.8% of 9873 patients without EC at recruitment subsequently developed this infection, particularly older patients and those with low CD4 counts.<sup>150</sup>

Oral thrush has many clinical presentations including: white, “cottage cheese” patches; a pseudomembranous type (a raw, bleeding surface when scraped); an erythematous type (flat, red, sometimes sore areas); *Candida* leukoplakia (non-removable white thickening of epithelium) and angular cheilitis (sore fissures at the corner of the mouth). Median rhomboid glossitis, a tongue abnormality associated with ovoid, denuded area in the median posterior portion of the tongue, has been associated with candidiasis. In AIDS patients such lesions are often spread over all intraoral surfaces and infection of the tongue may be severe enough to produce fissuring. In elderly patients, particularly those who wear dentures, a more chronic form of disease characterized principally by areas of non-specific erythema, often beneath denture surfaces, is seen.<sup>43</sup> Up to one-third of patients with EC may not have oral thrush<sup>151</sup> while more than two-thirds of patients with thrush have concomitant EC.<sup>152</sup>

Candidiasis can involve any site of the gut, but most commonly the oral cavity, esophagus and small bowel. These lesions may progress to hematogenous infection, obstruction<sup>153</sup> or even perforation.<sup>154,155</sup> The pathology of candidal infection of the lower gut ranges from mucosal ulceration with or without pseudomembrane to exophytic lesions. Pseudomembranes are composed of a mixture of yeasts and pseudohyphae embedded in necrotic debris and fibrin. Pseudohyphae may extend beyond the muscular layer and reach the serosa. Direct vascular invasion through the bowel wall has been reported in immunosuppressed patients receiving chemotherapy.<sup>156,157</sup> These patients may have extensive gut involvement – from mouth to anus – while non-neutropenic patients exhibit more localized disease.<sup>158</sup> Diagnosis rests on the presence of budding yeasts and/or pseudohyphae on KOH smears and fungal cultures along with a disrupted epithelium and a submucosal inflammatory reaction.

## *Candida* infections of the genitalia

*Candida* spp., particularly *C. albicans*, play a major role in VVC, the second most frequent genital complaint in women, with around 75% experiencing at least one episode in their lifetime, and half by 25 years of age.<sup>159</sup> Risk factors for VVC are frequently absent but severe forms may be associated with use of oral contraceptives, corticosteroids or antibiotics,<sup>160</sup> diabetes<sup>161</sup> and pregnancy.<sup>162</sup> Sexual transmission of *Candida* strains occurs between partners, especially in the setting of receptive oral sex,<sup>102,103,163</sup> as the infection does not appear to be transmitted through vaginal intercourse.<sup>102,105</sup>

In most cases the presentation is acute, the symptoms mild (pruritus, vaginal soreness, dyspareunia, external dysuria, and abnormal vaginal discharge) and rapidly responsive to short-course topical treatment (topical azoles better than nystatin). However, around 5% of women develop a recurrent vulvovaginal candidiasis (RVVC) (≥4 episodes in 1 year)<sup>164</sup> despite absence of underlying illness in most women. These infections are typically resistant to standard therapies. Vaginal cultures should be obtained to confirm the diagnosis of RVVC



**Table 8-2** Overview of Types of *Candida* infections and their predisposing factors

Type of candidiasis	Major predisposing/Risk factors
Hematogenous	Colonization: prolonged antibiotic use and long hospital stays Disruption of gut integrity: abdominal surgery with gut transfection, total parenteral nutrition Immunosuppression, systemic: extremes of age, malnutrition, immunosuppressive therapy, neutropenia, stem cell/solid organ transplantation, HIV infection with low CD4 counts, renal failure/hemodialysis, diabetes mellitus, severe burns
Oropharyngeal	Immunosuppression, systemic (same as under hematogenous candidiasis) Immunosuppression, local: radiotherapy for head and neck cancer, use of inhaled corticosteroids Local factors: dentures, especially if ill-fitting
Esophageal	Immunosuppression, systemic (same as under hematogenous candidiasis)
Vulvovaginal	Colonization: antibiotic use Immunosuppression, systemic: HIV infection, diabetes mellitus, systemic corticosteroids Other: oral contraceptives, pregnancy
Skin and nails	Local factors: moisture and occlusion, immersion of hands in water, peripheral vascular disease
Cutaneous congenital	Immunosuppression: prematurity Local factors: mother with intrauterine foreign body
Chronic mucocutaneous	Immunosuppression: T lymphocyte defects
Urinary tract	Immunosuppression, systemic: diabetes mellitus Local factors: indwelling urinary catheter, urinary obstruction, procedures on urinary tract
Pneumonia	Aspiration
Endocarditis	Local factors: major surgery, history of bacterial endocarditis or valvular disease, presence of prosthetic valve or long-term central venous catheter Other: intravenous drug abuse
Pericarditis	Immunosuppression, systemic Thoracic surgery
Central nervous system	Direct inoculation: surgery on the central nervous system, ventriculoperitoneal shunt
Ocular	Direct inoculation: ocular surgery or trauma
Bone and joint	Direct inoculation: surgery, trauma or intraarticular injections Diabetic foot infection
Abdominal	Local factors: solid organ transplantation, recurrent perforation, repeat abdominal surgery, anastomotic leaks, pancreatitis, continuous ambulatory peritoneal dialysis Immunosuppression, systemic: solid organ transplantation

and to identify unusual species, such as *C. glabrata* and other non-*albicans* species which account for 10–20% of patients with RVVC.

A local change in vaginal immune defenses appears more important than an impairment of systemic immunity<sup>165,166</sup> which may explain why the frequency of RVVC is not increased in HIV-infected patients with low CD4 counts.<sup>167</sup> Most RVVC are caused by the same strain of *Candida* that developed subtle genetic variations<sup>106</sup> rather than by drug resistance.<sup>165</sup>

Genital infections in males are less common than in females and can be caused by the yeast itself or by an allergic reaction

to *Candida* antigens after unprotected intercourse. *Candida* balanoposthitis presents as a mild irritation with focal erythema but may rarely worsen and cause phimosis.<sup>168</sup>

### **Candida infections of the skin and nails**

*Candida* spp. inhabit the skin and mucus membranes in around 75% of the population without causing harm.<sup>169</sup> However, candidal infection may develop in occluded body sites where the surface remains moist (groins, armpits, or spaces between toes and along breastfolds)<sup>170</sup> and present as a pruritic rash

with a poorly defined edge, abundant erythematous vesiculopustular lesions and interdigital fissures.<sup>170</sup>

Invasive infections of the fingernails (onychomycosis) are mainly caused by *C. albicans* and *C. parapsilosis* (less commonly *C. glabrata* and *C. guilliermondii*)<sup>171-173</sup> while dermatophytes are the most common cause of toenail onychomycosis.<sup>174,175</sup>

Chronic swelling and inflammation of the nailfold (paronychia) is a condition characterized by the presence, under the nailfold, of a mixed flora of normally commensal organisms. Yeasts and particularly *C. albicans* are typically part of this flora.<sup>170,176</sup>

Overall, cutaneous fungal infections are not uncommon in neonates. However, immunocompromised and premature neonates are susceptible to more serious infections.<sup>177</sup> Neonates may rarely develop cutaneous congenital candidiasis. Among neonates weighing >1000 g, the condition presents with a generalized macular erythematous rash that may become pustular, papular or vesicular, and subsequently desquamate. Among premature neonates weighing <1000 g, this entity presents with a widespread desquamating or erosive dermatitis that can evolve into hematogenous candidiasis and possibly death.

Skin lesions caused by *Candida* spp. can also represent a manifestation of hematogenous candidiasis and are of diagnostic value in neutropenic hosts.

Chronic mucocutaneous candidiasis is a rare condition in which susceptible individuals develop superficial *Candida* infections as a result of variable defects in T-lymphocyte responsiveness to the fungus. Through childhood and into adulthood, such patients suffer from unremitting mucocutaneous *Candida* lesions including severe nail involvement and vaginitis. The mucocutaneous lesions sometimes develop into a gross, disfiguring granulomatous appearance.<sup>178</sup>

## Candida infections of deep tissues

While single-organ *Candida* infections without concomitant disseminated disease may occur, most candidal deep tissue infections result from hematogenous candidiasis. Clinicians should therefore be alert to the possibility of disseminated infection even when only a single organ shows evidence of candidiasis.

### Urinary tract infections (UTI)

The isolation of *Candida* specimens from a urine sample represents contamination, colonization, and rarely lower or upper UTI. Contamination is more common in females with VVC and can be excluded by examination of a clean-catch urine sample. Colonization (asymptomatic candiduria) and infection occur in patients with the usual systemic risk factors (diabetes, immunosuppression, others) and in those with local risk factors such as presence of indwelling urinary catheter, urinary tract obstruction or instrumentation and surgery.<sup>179,180</sup> The prevalence of candiduria in hospitalized patients ranges from 2% to 11% and is highest among leukemia, BMT and ICU patients.<sup>181</sup> Differentiating colonization from infection may be difficult. Pyuria and a high urine colony count are not helpful for diagnosing infection in catheterized patients while the presence of >10<sup>3</sup> cfu/ml of *Candida* spp. may suggest infection among non-catheterized patients. A negative urinary culture cannot exclude infection. Asymptomatic candiduria usually has a benign course<sup>179,182</sup> although hematogenous

infections may be seen among severely immunosuppressed patients.<sup>183,184</sup>

In lower UTI (cystitis), symptoms are comparable to those observed with bacterial cystitis. If a cystoscopy is performed, soft, pearly white, elevated patches with hyperemic and friable mucosa underneath may be seen. Emphysematous cystitis and prostatic abscess are occasional complications of *Candida* cystitis.<sup>185</sup>

Upper UTIs are indistinguishable from bacterial pyelonephritis and urosepsis. These infections occur mainly among patients with urinary obstruction and stasis. Complications include pyonephrosis, focal abscesses, and fungal balls (bezoars) that may lead to obstruction, renal colic and papillary necrosis. Fungal bezoars are rare and develop mainly in the pelvis and upper ureters. Candidemia is an uncommon complication of ascending infection and occurs mainly among patients with obstruction, or following urologic manipulation. Ultrasonography and computed tomography scanning (CT scan) may diagnose fungal balls, hydronephrosis, and intrarenal and perinephric abscesses.<sup>185</sup>

Renal candidiasis is secondary to hematogenous candidiasis and presents with the usual manifestations of sepsis. Candiduria may be present without other symptoms of renal involvement other than reduction in renal function.<sup>185</sup>

### Pneumonia

Primary *Candida* pneumonia is very rare, and usually the result of aspiration,<sup>186</sup> in contrast to secondary *Candida* pneumonia, which is commonly seen in patients with hematogenous candidiasis.

### Empyema

*Candida* empyema is rare and typically occurs among patients with severe underlying diseases, particularly cancer. A preceding candidemia may be present. Symptoms are similar to those seen with bacterial empyemas and the diagnosis requires the isolation of *Candida* species from an exudative pleural effusion in a patient with clinical signs of infection. Bacterial pathogens may also be isolated. Treatment consists of systemic antifungal chemotherapy and closed drainage although mortality remains high because of the severe underlying illnesses. Intrapleural administration of antifungal agents may be considered.<sup>187</sup>

### Mediastinitis

*Candida* mediastinitis almost always occurs after thoracic surgery procedures (median of 11 days, range 6–100 days) and presents as chest wall erythema and/or drainage, fever, and sternal instability. The course may become complicated by contiguous or hematogenous spread. The mortality rate is >50%, in part because diagnosis may be delayed when intraoperative fungal cultures are not obtained or if candidal cultures are not taken seriously. Mediastinal drainage is critical to a successful outcome along with systemic antifungal chemotherapy.<sup>188-191</sup>

### Laryngitis or epiglottitis

Localized *Candida* laryngitis or epiglottitis is rare and may be life-threatening. Securing the airway is critical as is treatment with amphotericin B or an echinocandin.<sup>192</sup>

Oral fluconazole may be used in non-life threatening settings such as in non-neutropenic hosts.<sup>193</sup>

## Cardiovascular infections

**Endocarditis** Fungi account for 2–4 % of all fungal endocarditis and 65% of those are caused by *Candida* spp.<sup>194</sup> Among patients with prosthetic valve (PV) endocarditis,<sup>195</sup> 2–10% are candidal in origin; moreover, 25% of PV patients with candidemia develop PV endocarditis.<sup>196</sup> Finally, among IVDA, *Candida* spp. are responsible for 14% of all endocarditis.

Risk factors for *Candida* endocarditis include major surgery (cardiac and others), preexistent bacterial endocarditis or valvular disease and presence of in situ pacemaker or long-term CVC. Other populations at risk include neonates and occasionally immunosuppressed patients.

The clinical presentation of *Candida* endocarditis resembles bacterial endocarditis with fever (75%), new or changing heart murmur (50%), and/or heart failure (25%). Unlike bacteria endocarditis, however, the risk of embolization of major arteries is very high (more than two-thirds of patients) and may involve the brain, kidneys, spleen, liver, skin, eyes and the coronary arteries. The aortic and mitral valves are most commonly involved, even among IVDA and patients with CVC.<sup>197</sup>

**Myocarditis** This infection occurs almost always in immunocompromised patients with hematogenous candidiasis and is associated with conduction disturbances and shock.<sup>198-200</sup>

**Pericarditis** Pericarditis is rare but may lead to serious complications including hematogenous spread and tamponade.<sup>43,194,201</sup> Pericarditis often arises as a complication of thoracic surgery or contiguous spread from an adjacent focus, but hematogenous spread can occur. Risk factors include immunosuppression, thoracic surgery, pericardiectomy<sup>202</sup> and hematogenous candidiasis.<sup>203</sup> *Candida* pericarditis should be promptly treated with long courses of systemic antifungal agents and emergently pericardial decompression (pericardial window or pericardiectomy) if hemodynamic compromise is present or likely. Pericardiocentesis may be tried first if hemodynamic compromise is not impending.<sup>204</sup>

## Central nervous system (CNS) infections

CNS infections by *Candida* spp. are rare, and present as meningitis or abscesses, and occasionally as a stroke.<sup>205</sup> They are usually secondary to hematogenous candidiasis, or may result from CNS surgery or ventriculoperitoneal or ventriculoatrial shunt infection.<sup>43</sup> In patients with hematogenous candidiasis, meningitis is more frequent in neonates (64%)<sup>206,207</sup> than adults (15%)<sup>208</sup> and has a more indolent course in the latter population. Neurosurgery-related candidiasis resembles bacterial meningitis in adults. A lumbar puncture to obtain cerebrospinal fluid (CSF) for culture is essential to establish the diagnosis.

Similar to meningitis caused by tuberculosis or *Cryptococcus*, chronic *Candida* meningitis presents with headache, fever, and nuchal rigidity. Because the organism is present in low numbers, the yield of standard CSF cultures is poor and large-volume spinal taps are often required to obtain sufficient CSF for culture and diagnosis.

## Ocular infections

Ocular *Candida* infections include keratitis, chorioretinitis and endophthalmitis. Most cases of chorioretinitis and endophthalmitis are secondary to hematogenous candidiasis and may be its earliest manifestation. Occasionally, infections may result from trauma typically incurred during ocular

surgery. A recent study suggested a significant decrease in the incidence of ocular candidiasis over a 12-year study period together with a changing epidemiology, with up to one-third of infections now occurring in immunocompromised non-drug user patients compared to 100% among IV drug users reported previously.<sup>209</sup> Keratitis is usually associated with local trauma.

## Bone and joint infections

Most cases of bone and joint *Candida* infections are secondary to hematogenous candidiasis. Primary *Candida* osteomyelitis and septic arthritis are less common, and develop by contiguity in patients with infected diabetic foot ulcers or more commonly as a result of accidental implantation of the fungus by traumatic means (e.g., surgery, intraarticular injection of corticosteroids, or injection drug use). Osteoarticular infections may not become symptomatic for several months after an episode of candidemia or a surgical procedure and symptoms are typically more subtle than with bacterial osteoarticular infections. Hence, delays in diagnosis, especially in patients with vertebral osteomyelitis, are not uncommon. In primary septic arthritis, the infection typically involves a single joint and may involve native as well as prosthetic joints. Local symptoms of pain on weight bearing or on full extension and decrease in range of motion may be present. The site of involvement among patients with osteomyelitis varies with age, with vertebrae most commonly involved in adults compared to involvement of the long bones in children. Local pain is the usual presenting symptom. Diagnosis is achieved by culturing the organism from the joint fluid. Even a single colony of *Candida* on culture of joint fluid should be taken seriously. The diagnosis of candidal osteomyelitis is more difficult

## Abdominal infections

*Candida* spp. are frequently recovered from intraabdominal samples but should only be considered as serious infections (abscesses, peritonitis or hematogenous candidiasis) in certain settings such as among patients with recurrent perforations, necrotizing pancreatitis, failure of previous abdominal infection to respond to therapy and anastomotic leakages.<sup>210-213</sup>

Biliary candidiasis (infection of gallbladder and/or the biliary tree) is very rare.<sup>214-216</sup> Typically, patients have candidemia and/or several risk factors for hematogenous candidiasis. It is thus likely that biliary candidiasis is secondary to hematogenous spread.<sup>217,218</sup> Gangrenous cholecystitis and obstruction to the common bile duct with a *Candida* fungus ball have also been described.<sup>219,220</sup>

The diagnosis is made when pure or persistent growth of *Candida* spp. occurs on biliary tract secretions. Histopathologic documentation of tissue invasion is desirable but not always feasible.

There is an increasing appreciation of the role of *Candida* infections following acute pancreatitis,<sup>221</sup> with a large series of patients undergoing surgery for infected pancreatic necrosis reporting presence *Candida* spp. in 10% of patients.

Hepatic, gallbladder, and subphrenic abscesses have also been described in cancer patients with percutaneously placed drainage catheters.<sup>222</sup>

Fungi account for 8% of peritonitis occurring in patients on continuous ambulatory peritoneal dialysis (CAPD),<sup>223</sup>



with *Candida* spp. (mostly *C. albicans*) causing 75% of infections.<sup>224</sup> *Candida* peritonitis has also been reported in patients with liver cirrhosis<sup>225</sup> and intraabdominal malignancies.<sup>226</sup> *Candida* peritonitis presents with low-grade fever, abdominal pain, and tenderness. The peritoneal dialysate is usually cloudy and contains >100 neutrophils/mm<sup>3</sup>. Untreated, *Candida* peritonitis may lead to hematogenous dissemination and abscess formation, requiring surgical drainage.<sup>212</sup> The diagnosis is made by aspiration of fluid under computed tomography (CT) or ultrasound guidance or at the time of surgery. Culture of *Candida* species from an indwelling drain is not adequate for the diagnosis of infection, since it often reflects only colonization or contamination of the drain.

### Wound infections

The diagnosis of candidal wound infections is difficult. Recovering *Candida* spp. from wounds does not necessarily imply that the organism is causing tissue infection and should not compel physicians to use antifungal therapy.

## Hematogenous candidiasis

### Incidence

Several surveys have confirmed the increasing rate of candidemia worldwide during the 20th century,<sup>124,227-229</sup> peaking in the late 1980s only to decrease after the introduction of fluconazole. This decrease has been observed in a tertiary care community hospital,<sup>229</sup> among leukemia<sup>123</sup> and ICU patients<sup>227</sup> and in an international surveillance that included patients from 250 centers in 32 countries.<sup>230</sup>

Data from the nationwide US SCOPE program (1995–2002) show that *Candida* spp. continue to be the fourth most common cause of nosocomial BSI (9% of all BSI)<sup>2</sup> and the third most common cause of ICU BSIs accounting for >10% of 10,515 episodes.<sup>2</sup>

In population-based studies, the incidence of candidemia varies from 8–10/100,000 in the US<sup>231,232</sup> to 1.81/100,000<sup>233</sup> and 1.4–4.9/100,000<sup>234-236</sup> in Australia and Europe, respectively.

The incidence of candidemia also varies among patient populations: 22–38% among total parenteral nutrition (TPN) recipients<sup>237,238</sup> and 1.8–7.6% of burn unit admissions, increasing to 12–21% among burn patients colonized with *Candida* strains, and to >34% when >2 sites are colonized.<sup>239-243</sup> Prior to the introduction of fluconazole, candidiasis was the most common invasive fungal infection (15–20% incidence) in BMT recipients<sup>244-246</sup> but has declined since.<sup>247,248</sup>

### Mortality and outcome predictors

Wide variations exist in the attributable mortality of candidemia, ranging from 5% to 71%,<sup>124,233,235,248-255</sup> and are probably a result of differences in type and size of populations studied, disease definitions, time endpoint for evaluation and treatment strategies. In one study, older age, acute renal failure and unfavorable APACHE II scores were independent predictors of mortality. Of interest, candidemia was not associated with increased mortality after adjustment for these factors.<sup>256</sup>

Several key prognostic factors for outcome (mortality) have been identified in several studies that relied on a validated severity of illness score and performed multivariate analysis. Poor outcome predictors generally included older

age, high severity of illness scores, visceral dissemination and persistent neutropenia. Lack of CVC removal was identified as an independent prognostic factor in only one of these 10 studies.<sup>250,256-264</sup>

A more recent study reported an overall mortality of 44%. Early death (day 3–7 after candidemia) was independently associated with hematologic malignancy (while antifungal therapy and removal of CVCs were protective). Intubation was associated with higher odds for late death.<sup>236</sup> The study, however, excluded patients with *C. parapsilosis* BSI (though *C. parapsilosis* is most frequently associated with CVC) and patients who died during the first 2 days. More importantly, severity of illness score was not included as an outcome variable despite the markedly higher mortality among patients with high score (80%) compared to those with intermediate or low scores, 39% and 29%, respectively. In addition, a non-validated model for severity of illness was used to perform some of the analyses.

Another retrospective study described the outcome of 404 patients with candidemia and classified outcome according to whether candidemia was CVC related or not.<sup>265</sup> Overall, CVC removal did not influence outcome but did so among the smaller subset of patients with “CVC-related” candidemia along with absence of visceral dissemination, no recent chemotherapy or corticosteroid therapy, and good response to therapy. Surprisingly, persistent neutropenia and severity of illness score, both previously shown by the same investigators to be independent prognostic factors on a large subset of the same patient population,<sup>266-271</sup> were not included in the analysis of prognostic factors.

Another study of 853 candidemias identified older age, ICU stay, sepsis at diagnosis, corticosteroid therapy, neutropenia, hemodialysis and TPN as important predictors of death. Removal of a CVC was not protective while antifungal treatment was.<sup>233</sup> A study of 249 episodes of candidemia in cancer patients identified several factors predictive of 30-day mortality: older age and severe underlying disease, and among hematology patients, allogeneic BMT, septic shock and lack of antifungal prophylaxis.<sup>251</sup>

Taken together, important factors associated with increased mortality in most studies include older age, poor physiologic score (including organ dysfunction, severity of illness scores), visceral dissemination and immunosuppression (neutropenia and corticosteroid therapy). Antifungal therapy appears protective while CVC retention is not.<sup>272</sup>

### Risk factors

Three factors contribute to the development of hematogenous candidiasis.<sup>273-275</sup>

1. Increased colonization by *Candida* spp. as a result of endogenous or exogenous factors.
  - *Endogenous*: prolonged antibiotic therapies suppress the endogenous microflora<sup>276</sup> and enhance the overgrowth of endogenous *Candida* spp. at mucosal sites.<sup>277,278</sup>
  - *Exogenous*: prolonged hospital stay increases the risk of acquisition of *Candida* strains from healthcare workers or the hospital environment.<sup>279</sup> Intensive care unit length of stay is an important risk factor, with the rate of infections rising rapidly after 7–10 days.<sup>111,280,281</sup>

2. Damage to the integrity of the gut mucosa leading to increased fungal translocation as observed with TPN,<sup>238</sup> malnutrition,<sup>282</sup> surgery,<sup>283</sup> chemotherapy-induced mucositis,<sup>284,285</sup> severe burns,<sup>286,287</sup> and graft-versus-host disease (GvHD).<sup>288</sup>
3. Immunosuppression, local (increased candidal overgrowth and/or translocation) or systemic (dissemination). Conditions that suppress T cell and/or phagocytic immunity include prematurity, severe burns,<sup>289-291</sup> hemodialysis,<sup>292,293</sup> TPN,<sup>294,295</sup> cancer (especially hematologic), neutropenia, AIDS,<sup>296,297</sup> immunosuppressive therapy such as steroids, cancer chemotherapy and receipt of BMT or SOT.

The presence of CVC is reported as a risk factor for candidemia in some,<sup>298-302</sup> but not all studies.<sup>277,303</sup> In a recent multicenter cohort study of 1699 ICU patients (including 79 with invasive candidiasis), multifocal candidal colonization, TPN, surgery and severe sepsis independently increased the risk of candidemia while presence of CVC did not.<sup>304</sup>

The CVC is thought to be a risk factor for candidemia as a result of *Candida* skin contamination at CVC sites which is alleged to cause CVC infection and subsequent dissemination. However, and in contrast to data supporting gut colonization by *Candida* spp. as a source for candidemia, data to support the skin hypothesis as primary source are not available.<sup>275</sup> In addition, *Candida* spp. that predominate in skin samples are *C. guilliermondii* and *C. parapsilosis* (among the least common causes of candidemia) and the half-life of *Candida* spp. on the skin is only a few minutes. Moreover, studies that identified CVC as a risk factor for candidemia did not typically include a validated severity of illness score. In these studies, presence of CVC represented more a marker of severity of illness rather than a risk factor for candidemia. However, CVCs are thrombogenic and thrombi may occasionally get infected during hematogenous candidiasis and may become a source of persistent infection.<sup>305-307</sup>

### Clinical presentation

**Acute disseminated candidiasis** In neonates, acute disseminated candidiasis (ADC) is most commonly caused by *C. albicans* and its clinical presentation is different from that in adults.<sup>308</sup>

In a prospective study comparing candidemia in children and adults, septic shock and meningitis occurred more commonly in children.<sup>309</sup> Spread to different organs is common,<sup>310</sup> including two-thirds to skin (66%)<sup>311</sup> or CNS (64%)<sup>207</sup> and 50% to the retina,<sup>312</sup> though empiric antifungal treatment has markedly decreased the incidence of endophthalmitis to 6%.<sup>313</sup> Respiratory dysfunction and apnea are also common (70%).<sup>314</sup>

Among adults, ADC occurs in non-neutropenic ICU patients and in neutropenic patients with hematologic malignancies receiving antineoplastic chemotherapy.

The infection can involve any organ including the eye. The frequency of ocular involvement by *Candida* spp. varies from 3%<sup>315</sup> to 78%<sup>316-319</sup> depending on the population studied (less in neutropenic patients, probably due to their inability to mount an inflammatory response), the diagnostic criteria used, the study design (prospective versus retrospective) and the clinician performing the examination (ophthalmologist versus non-ophthalmologist). Ocular involvement may be present at diagnosis of candidemia or may not develop until later during

the course of the infection,<sup>320</sup> implying that patients with candidemia should undergo a dilated retinal examination by an ophthalmologist not only at baseline but also 2 weeks after diagnosis. The infection can be sight threatening if it is not promptly treated. The primary presenting symptoms of ocular involvement are pain and a gradual decrease in visual acuity. Classic findings of chorioretinal involvement are focal, glistening, white, infiltrative, often mound-like retinal lesions. In the event of vitreous extension, a vitreal haze or fluffy white balls or “snowballs” in the vitreous may be seen.

Among neutropenic patients, skin lesions develop in 10–25% (Fig. 8-5) sometimes with myalgias resulting from muscular abscesses.

Lesions may appear suddenly as clusters of painless small pustules covering any area of the body. They may evolve into larger nodules,<sup>321,322</sup> with necrotic centers as with ecthyma gangrenosum.<sup>323</sup> In severely neutropenic patients, skin lesions may be macular without any pustules, resembling non-specific drug rash, because of absence of inflammatory reaction in the setting of profound neutropenia.<sup>324,325</sup>

Diagnosis can be made by scraping the base of a pustule with a scalpel blade, which shows yeast on Gram stain, and by culture. Punch biopsies of skin lesions can also be performed for histopathology and culture.

In addition, signs of multiorgan system failure may be present in patients with hematogenous candidiasis.

Lung involvement can occur during ADC and the most common CT scan findings may be indistinguishable from those caused by invasive aspergillosis, including multiple bilateral nodular opacities often associated with areas of consolidation,<sup>326</sup> the CT halo sign, cavitation and ground-glass opacities.<sup>327</sup>

**Chronic disseminated candidiasis** Less common than the acute disseminated disease, chronic disseminated candidiasis (CDC) (previously known as hepatosplenic candidiasis) is almost always associated with recovery from neutropenia and may arise subsequent to an episode of ADC. Because blood cultures are not commonly positive, candidal invasion through the portal vasculature has been proposed as another mechanism by which CDC may develop. The condition occurs mainly among patients with acute leukemia undergoing cytotoxic chemotherapy, and



**Figure 8-5** Erythematous papular lesions developed throughout the body. ([www.medscape.com](http://www.medscape.com))

is characterized by persistent fever unresponsive to antibiotics, negative blood cultures (usually), abdominal pain (mainly right upper quadrant), nausea, vomiting, and anorexia, increased liver function tests, in particular serum alkaline phosphatase, and presence on radiologic imaging of multiple abscesses in liver and at times in spleen, lungs and kidneys. Hepatomegaly and/or splenomegaly is present in half of the patients while abdominal tenderness can be elicited in about two-thirds.<sup>328</sup>

*Candida* abscesses are detectable by CT scan, magnetic resonance imaging (MRI) or most commonly by ultrasonography (Fig. 8-6). Four ultrasonographic patterns of CDC have been described. Early in the disease, the *Candida* microabscesses may show a “wheel within a wheel” image (first pattern) or a “typical bull’s eye” (second pattern) and/or uniformly hypoechoic lesions (third pattern). Late in the course, fibrosis or calcification of the lesions may show echogenic foci with variable degrees of acoustic shadowing (fourth pattern). On CT scan, only the third and fourth patterns are commonly seen.<sup>329</sup> MRI is more sensitive than CT<sup>330</sup> with three MRI patterns described:

- acute (within 2 weeks of therapy); <1 cm lesions best shown as well-defined, high-intensity foci on T2-weighted images
- subacute (after 2–12 weeks of therapy); similar size lesions best observed as mildly hyperintense on T1-weighted images, along with a perilesional ring
- chronic (later in the disease process); 1–3 cm lesions with irregular margins with decreased enhancement after gadolinium.<sup>331</sup>

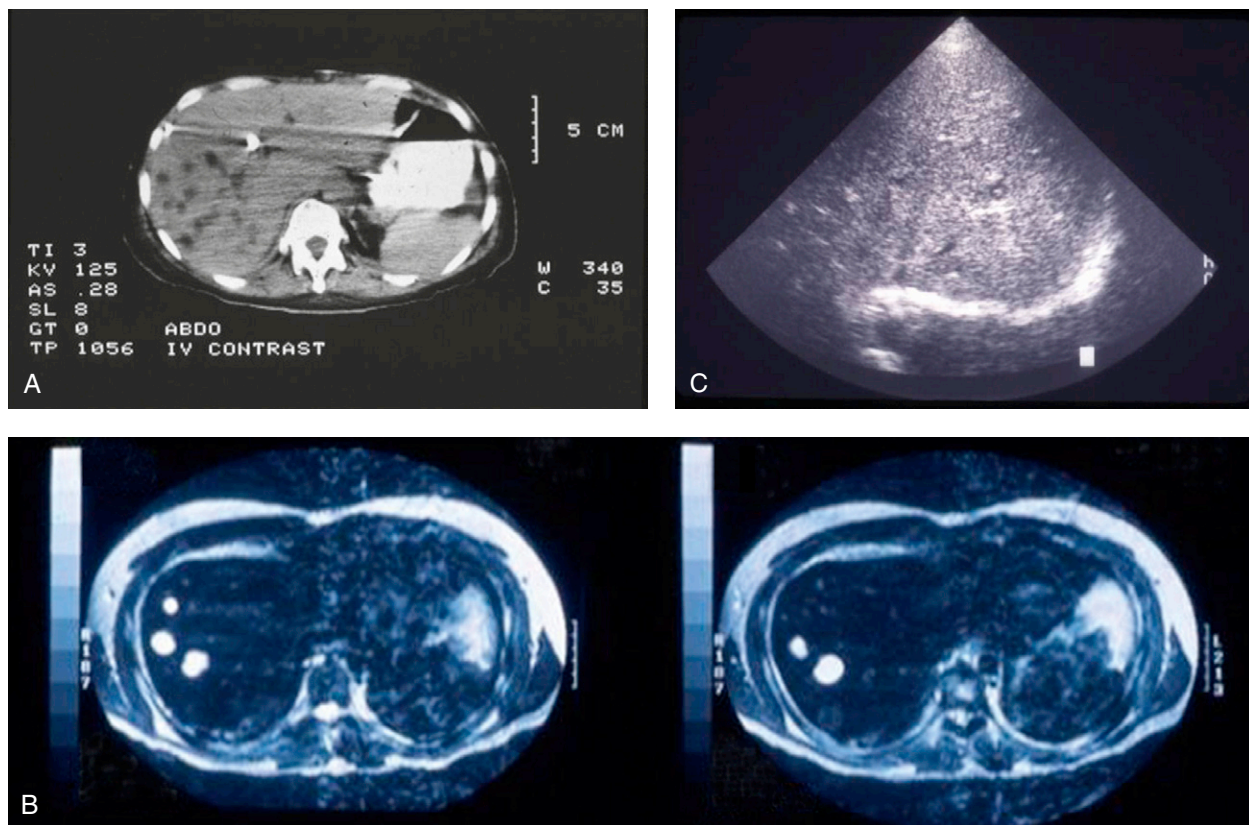
A unique pattern of organ involvement is seen among IVDA who develop ADC following IV injection of contaminated heroin solutions. The initial symptoms may last from a few hours to a month, and consist of fever, shivering, sweating, asthenia, or headache.<sup>332</sup> Within 1–4 days after candidemia, >75% of patients develop nodular cutaneous lesions affecting mainly the scalp<sup>333,334</sup> followed by ocular involvement in 50% of patients (chorioretinitis, hyalitis, episcleritis, anterior uveitis, and endophthalmitis), with osteoarticular lesions (costochondritis and vertebral lesions) in more than one-third of patients appearing last (as late as 5 months after candidemia).

## Catheter-related candidemia

The term catheter-related candidemia implies that the catheter plays a key role in the pathogenesis of candidemia, either as a primary source of the organism (primary catheter-related candidemia as a result of CVC colonization from skin) or as a nidus that can perpetuate a candidemia that had originated from another site (secondary catheter-related candidemia as a result of CVC seeding from the blood).

### Primary catheter-related candidemia

Catheter-related candidemia is defined as candidemia in a patient with an intravascular catheter, and no other obvious origin for the infection after careful clinical and laboratory evaluation. If the catheter is removed, a quantitative tip culture should recover  $\geq 15$  cfu of the same *Candida* spp. by the roll



**Figure 8-6** Liver *Candida* abscesses in patients with chronic disseminated candidiasis. (A) CT scan of liver showing multiple hypoechoic lesions. (B) MRI of the liver: note high-intensity foci of infection. (C) Ultrasound of the liver: bull's eye shown by arrow.



plate method or  $\geq 100$  cfu by the sonication technique. If the catheter is not removed, a quantitative blood culture collected through a CVC should contain at least a 10-fold greater concentration of *Candida* spp. than a simultaneously collected quantitative peripheral blood culture.<sup>335</sup>

Because sources other than CVC (gut mostly, occasionally contaminated TPN solutions) are the primary source of candidemia in a large proportion of patients, the definition of catheter-related candidemia should also include lack of recovery of the same *Candida* spp. from other sites, particularly the gut. In addition, and in light of the novel findings of the genotypic diversity of *Candida* spp., the definition should also include molecular relatedness between colonizing (skin, gut and CVC tip) and infecting strains (blood), as the mere recovery of the same *Candida* spp. from CVC tip and blood cannot imply that these organisms are genotypically related.

As mentioned under risk factors for hematogenous candidiasis, the gut, not the skin, is the primary source of most hematogenous candidiasis. Thus, primary catheter-related candidemia is rather uncommon.

#### Secondary catheter-related candidemia/septic thrombophlebitis

In this entity, the primary source of the candidemia is gut translocation of *Candida* spp. and not the catheter. During hematogenous spread, *Candida* spp. adhere to a CVC-associated thrombus, the vessel walls and the CVC, leading to septic thrombophlebitis. Hence, the catheter has now become a source from which *Candida* spp. can subsequently disseminate and hence the term secondary catheter-related candidemia.

*Candida* thrombophlebitis of the central veins is rare<sup>307,336,337</sup> and develops at sites of CVC insertion and dwelling, i.e., the subclavian, innominate and superior cava veins. In most cases, *C. albicans* is the causative pathogen and the risk factors are similar to those for hematogenous candidiasis. The clinical findings are those of sepsis, along with local findings of edema and/or pain of the area involved, and persistent candidemia (2–3 weeks) even after CVC removal and appropriate antifungal chemotherapy.<sup>307</sup>

*Candida* thrombophlebitis of the peripheral veins is also rare and with similar clinical presentation except that local findings are more obvious, ranging from a non-inflamed thrombosed vein to a warm, tender, erythematous vein with or without purulent drainage.<sup>305,306,338–340</sup> That septic thrombophlebitis may present as a non-inflamed thrombosed vein suggests that the vascular catheter may become infected following hematogenous candidiasis and does not support the hypothesis that candidemia in patients with septic thrombophlebitis results from skin contamination and primary catheter infection.

## Diagnosis of candida infections

Because blood cultures may be negative in a significant number of patients with IC, the diagnosis frequently rests on a combination of clinical and laboratory findings. Clinically helpful clues in a patient at risk include the presence of unexplained fever or sepsis and metastatic skin lesions (in neutropenic hosts only). In such patients, complete physical examination should be repeated in a search for a potential focus (intraabdominal, other), for the presence of skin lesions, myalgias and muscular abscesses and/or endophthalmitis. Laboratory diagnosis

of IC depends upon three approaches – microbiologic, histopathologic and immunologic – and on close collaboration between clinicians, microbiologists, and pathologists.

### Direct microscopy

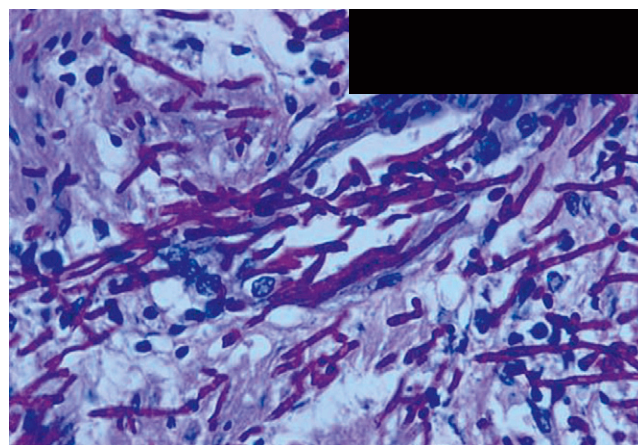
Direct microscopic examination of clinical material with wet mounts, Gram, Giemsa, periodic acid-Schiff (PAS) or Calcofluor stains can rapidly identify the characteristic combination of budding yeast cells and pseudohyphae seen with *Candida* although *Trichosporon* and *Geotrichum* may produce similar findings. In patients with suspected high-grade candidemia, an early diagnosis can be made by examination of blood smears,<sup>341</sup> particularly if the microscopist is specifically asked to look for yeasts (Wright–Giemsa stain, 200 high-power field).<sup>342</sup>

Cytologic and histologic stains, including the hematoxylin and eosin (H&E), GMS and PAS stains, can detect *Candida* spp. in various fluids and tissues (Figs 8-7, 8-8). However, the H&E stain may be inadequate when there are few fungal elements. In such settings, GMS and PAS stains can detect smaller numbers of fungi and better delineate their morphology. When needed, *Candida*-specific immunofluorescent stains can confirm a presumptive histologic identification.

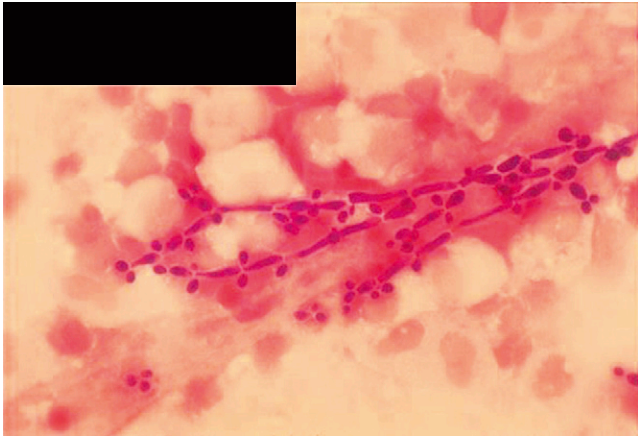
### Microbiology

The low sensitivity of blood cultures for detecting candidemia has been significantly improved with newer technologies such as the continuous monitoring automated blood culture systems (particularly the BactT/Alert (BioMerieux) and the Bactec® (Becton-Dickinson) systems) and the lysis-centrifugation method (Isolator, Wampole).<sup>343,344</sup>

Isolation of *Candida* from blood requires preliminary broth culture to amplify the usually low numbers of yeasts, which can then be plated on other media for isolation and identification. Biphasic blood culture media and vented culture bottles are optimal for detection of *Candida* yeasts and pre-treating blood samples by cell lysis and centrifugation greatly enhances the yield.<sup>345</sup> Combination of lysis-centrifugation and



**Figure 8-7** Direct smear of urine from a patient with candidiasis of the kidney showing *C. albicans* in mycelial or tissue phase with blastoconidia budding from the pseudohyphae. (Courtesy of the Geraldine Kaminski Medical Mycology Library. Produced by David Ellis and Roland Hermanis. Copyright© 2003 Doctorfungus Corporation.)



**Figure 8-8** PAS-stained section of postmortem esophagus showing invasion of blood vessel by *C. albicans*. Note blastoconidia and branched pseudohyphae. (Courtesy of the Geraldine Kaminski Medical Mycology Library. Produced by David Ellis and Roland Hermanis. Copyright© 2003 Doctorfungus Corporation.)

the automated Bactec® identification system offers an average time to detection of 3–4 days for *C. albicans*, *C. parapsilosis* and *C. tropicalis*, whereas *C. krusei* and *C. glabrata* often take longer to grow. Therefore, blood cultures obtained to rule out hematogenous candidiasis should be evaluated up to 2 weeks.

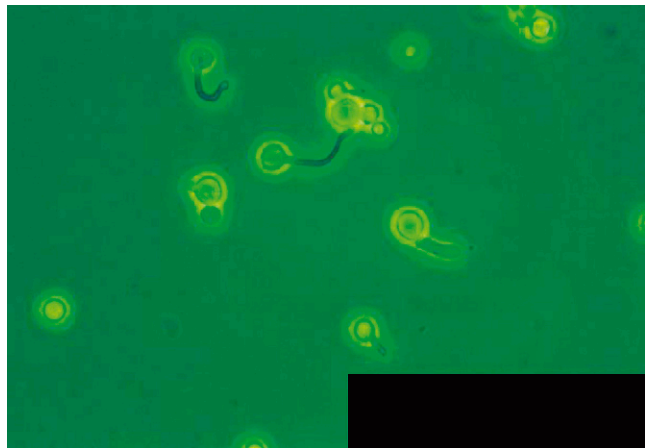
Sampling various anatomic sites (throat, sputum, stools, urine, others) for candidal colonization is useful to identify risk categories for IC (low if persistently negative cultures<sup>346-350</sup> versus high if multifocal colonization and/or *C. tropicalis* colonization in immunocompromised patients)<sup>111,347,348</sup> and those in whom antifungal therapy with agents other than fluconazole should be considered (colonization with *C. glabrata* or *C. krusei*).<sup>111,119,351</sup>

*Candida* spp. grow well on standard mycologic media at 35°C, such as Sabouraud agar pH 5.6 with chloramphenicol and gentamicin, and on many bacterial media, including blood agar, brain-heart infusion agar and tryptose agar. Cycloheximide (Actidione) inhibits candidal growth and hence should not be incorporated in the laboratory work-up for *Candida*. The time needed for species identification can be shortened by using media that can differentiate *Candida* spp. by colony color such as CHROMagar Candida® (Fig. 8-9), which inhibits bacterial growth and provides presumptive identification of *C. albicans*, *C. tropicalis*, *C. dubliniensis*, and *C. krusei*. Fongiscreen 4H® also allows similar detection. Other culture media that allow rapid identification of *C. albicans* include CandiSelect®, Sanofi Diagnostic Pasteur, France; Fluoroplate® Candida, Merck, Germany; Murex Candida albicans®, Murex Diagnostic, USA; and Albicans ID®, BioMerieux, France.

The most common strategy for identification of yeasts is to start with rapid, simple, and specific tests to identify *C. albicans* since this single species accounts for the majority of yeasts grown from clinical samples. This identification is easily done on any of the isolation media described above. Alternatively, identification of *C. albicans* could rely on this species' ability to produce germ tubes in serum after 3 hours at 37°C (>90% of *C. albicans* isolates produce germ tubes) (Fig. 8-10).



**Figure 8-9** Differentiation of *Candida* species by isolation on CHROMagar Candida®. The green colonies are *C. albicans*; *C. tropicalis* appear as blue-gray colonies and *C. krusei* as pale colonies. Only *C. albicans*, *C. krusei* and *C. tropicalis* can be dependably recognized on this medium: other species give colonies ranging from a very pale to a dark pink color. (Courtesy of Dr Santosh Karade, Dr Karishma Kaushik, Department of Microbiology, Armed Forces Medical College, Pune, INDIA. Copyright© 2007 Doctorfungus Corporation.)



**Figure 8-10** Screening test for the identification of *C. albicans*. Production of characteristic germ tubes by *C. albicans* in serum or plasma after 2–3 hours' incubation at 37°C. (Courtesy of the Geraldine Kaminski Medical Mycology Library. Produced by David Ellis and Roland Hermanis. Copyright© 2003 Doctorfungus Corporation.)

However, germ tube production is also seen with *C. dubliniensis* and *C. stellatoidea*. *C. dubliniensis* closely resembles *C. albicans* in screening tests. Only routine testing of presumed *C. albicans* isolates for absence of  $\beta$ -glucosidase activity<sup>352</sup> can confirm that the isolate is *C. albicans* without need for DNA fingerprinting. Isolates that cannot be recognized as *C. albicans* or *C. dubliniensis* should be examined for the morphology of

their blastoconidia and for their ability to produce pseudohyphae and chlamydoconidia on suitable semi-starvation media such as corn meal or cream of rice-Tween agars. A trehalose test can identify *C. glabrata*,<sup>353</sup> as can the differential growth on eosin methylene blue agar (rapid growth, versus no growth or slow growth on blood agar).<sup>354</sup> Most laboratories continue to rely on a battery of biochemical tests offered in the API-20C strip for yeast identification or a more extended kit ID 32C, API Candida kit.

Recently, a molecular method has been developed that uses a fluorescein-labeled, peptid nucleic acid probe that targets *C. albicans* 26S rRNA for identification of yeasts directly from blood culture bottles (*C. albicans* PNA FISH™ test, AdvanDx Inc, Woburn, MA). The method allows for very rapid identification (within 2–3 hours) of *C. albicans* versus non-*albicans* species provided the blood culture bottle tests positive and yeasts are visible on Gram stain. The test is highly sensitive and specific regardless of the blood culturing system or broth formulation used.<sup>355–358</sup> Applying this method results in identification 24–48 hours earlier than with conventional methods and may offer substantial savings as a result of more directed antifungal therapy.<sup>359</sup>

## Non-culture methods

Extensive work has been done on non-culture methods for diagnosis of hematogenous candidiasis, with somewhat disappointing results. The only promising approaches appear to be serial testing of the combination of the Platelia *Candida* antigen test (detects mannan) with the Platelia antibody test (detects antimannan antibodies)<sup>360,361</sup> and the  $\beta$ 1-3-glucan (BG) test (Fungitell® BG assay, Associates of Cape Cod Inc, Falmouth, MA). BG is a component of the cell wall of most fungal pathogens including *Candida* and can be detected and quantitated in the bloodstream of patients with fungal infections and has a sensitivity and specificity >90% in patients with hematogenous candidiasis.<sup>362–367</sup>

Limitations of the BG test include false-negative results (if hyperbilirubinemia or hypertriglyceridemia presents) and false-positive results. False positives can result from excess sample manipulation and exposure to gauze or other materials that contain glucans, or in presence of Gram-positive bacteremia, hemolysis, hemodialysis with cellulose membranes and therapy with IV immunoglobulins and albumin.<sup>363,368</sup> Minimal sample manipulation and two sequential positive results improve test performance.

Use of the polymerase chain reaction for the diagnosis of IC remains limited by lack of standardization of testing protocols and limited clinical validation.

## Management of hematogenous candidiasis

The management of localized superficial or deep invasive *Candida* infections will be discussed in the corresponding chapters (e.g., for management of *Candida* vaginitis, see Chapter 26 on genitourinary fungal infections). Because most deep single-organ candidiasis arises secondary to bloodstream candidal infection, these infections should be treated like hematogenous candidiasis.

## Acute disseminated candidiasis

### Empiric therapy

Because current tools are insufficient for early diagnosis, and as mortality of ADC increases with treatment delays and because fluconazole prophylaxis appears to decrease the incidence and mortality of ADC,<sup>229,230</sup> empiric therapy with fluconazole is commonly applied (Fig. 8-11).

Hence, identifying patients at high risk for ADC and who may benefit from early therapy is critical. In general, recovery of *Candida* spp. from sterile sites (blood, peritoneal fluid in a patient with peritonitis, or other sites) in a patient with a compatible clinical picture is diagnostic of invasive candidiasis.<sup>85,211</sup> In addition, recovery of *Candida* species from multiple sites in patients with unexplained fever, leukocytosis, and hypotension should be considered highly suggestive of invasive candidiasis and empiric therapy should be commenced.

Scoring systems have been developed to predict such high-risk patients who would be appropriate candidates for early antifungal therapy. These systems include a fungal colonizing index in which the greater the number of positive sites, the greater the increased risk for invasive infection,<sup>369</sup> and a combination of colonization and other risk factors<sup>304</sup> in which score components include severe sepsis, +2.038, multifocal colonization by *Candida* spp., +1.112, surgery, +0.997, and TPN, +0.908; patients with >2.5 score are seven times more likely to have invasive candidiasis. Another scoring system relies on variables other than colonization including  $\geq 4$  days in the ICU, CVC, diabetes mellitus, new hemodialysis, TPN, and broad-spectrum antibiotics.<sup>370</sup> Choices include an azole, an echinocandin or an amphotericin B (AmB) preparation (see Fig. 8-11).

If an azole is selected, fluconazole (400–800 mg/day) is preferred unless local epidemiology suggests a high likelihood of non-*albicans* species, in which case voriconazole would be more appropriate (voriconazole is active against *C. krusei* and some *C. glabrata*). Empiric fluconazole is thought to be cost-effective in the ICU setting when the likelihood of invasive candidiasis is >2.5% or when fluconazole resistance is <24%.<sup>371</sup> If higher levels of fluconazole resistance are likely, an echinocandin is preferred. Clinical features do not allow for accurate prediction of the infecting species.<sup>372</sup>

### Therapy of documented infection

If an organism is recovered from the bloodstream, prompt identification at the species level should be made and antifungal therapy adjusted accordingly. In typical settings, species identification may take several days but may be significantly shortened with use of PNA FISH (see Fig. 8-11).<sup>355–358</sup>

The choice of empiric antifungal agent is based on several factors, including susceptibility of the infecting species, hemodynamic status, potential for resistant strains (local hospital epidemiology and prior exposure to antifungal agents) and cost-effectiveness (Tables 8-3–8-5).

Choices of therapy include fluconazole, voriconazole, the echinocandins (casposungin, micafungin, anidulafungin), Amphotericin B deoxycholate (D-AmB) or its lipid formulations.<sup>373</sup> Dosage schedules of the various agents are given in Table 8-4.

**Azoles** Fluconazole is a well-tolerated triazole, with good activity against *Candida* spp. except *C. krusei* and *C. glabrata*. All five studies that compared fluconazole (200–800 mg/day)



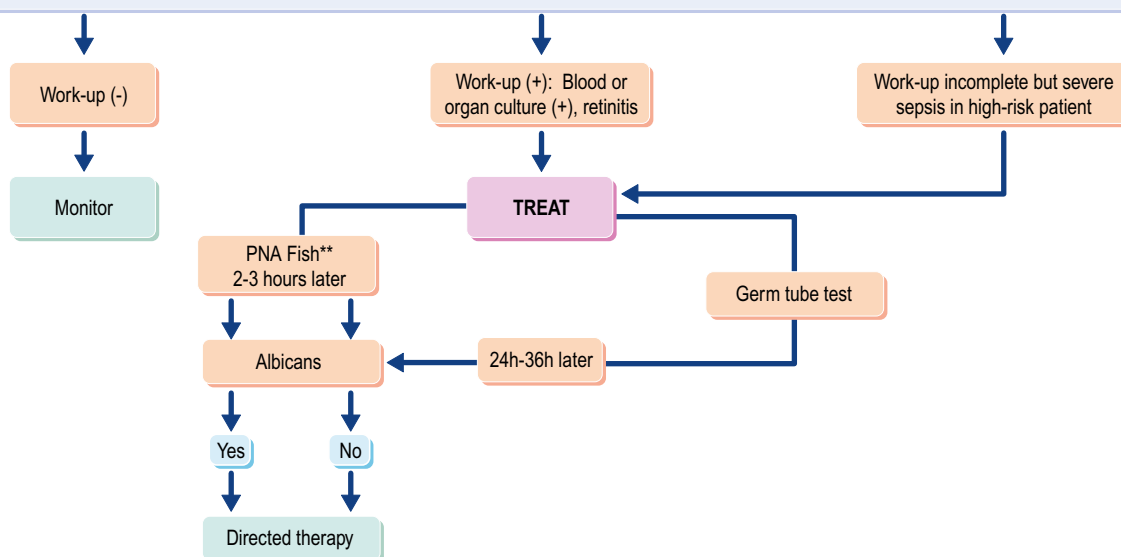
**Multifocal Candida colonization ( $\geq 2$  sites) and presence of one or more major risk factors\*:**

-Increased candidal translocation across the gut:  $> 3$  antibiotics, total parenteral nutrition, major surgery especially liver transplantation, malnutrition, major trauma, severe burns, chemotherapy-induced mucositis, graft-versus host disease.

-Immunosuppression: premature neonates, hemodialysis, cancer (especially hematologic), neutropenia, immunosuppressive therapies such as steroids, cancer chemotherapy, bone marrow or organ transplantation (especially liver), AIDS, severe burns, Central venous catheters found to be a risk factor for candidemia in few series

Fever of unknown etiology unresponsive to broad-spectrum antibiotics.

- Culture urine, wound, drain sites and fluids, throat and stool
- Blood cultures (3-4 sets over 36-48 h), using optimal blood culturing system
- Physical examination: abdominal focus, thrombophlebitis, retinitis (mainly *C. albicans*), multiple nodular skin lesions (in neutropenic patients).
- Exclude other diagnoses as cause of persistent fever on antibiotics



**EMPIRIC THERAPY:** (agents listed by order of preference).

**Hemodynamic instability:** echinocandin or lipid Amphotericin-B (better tolerated than Amphotericin-B).

**Hemodynamically stable:** fluconazole, echinocandin or voriconazole.

Incorporate knowledge of local hospital epidemiology in treatment decision.

Avoid fluconazole if recent exposure of patient ( $< 30$ -60 days) to fluconazole.

Voriconazole effective against *Candida Krusei*.

**DIRECTED THERAPY:** (agents listed by order of preference).

*C. albicans*, *C. tropicalis*: fluconazole, echinocandin, voriconazole, lipid amphotericin-B.

*C. krusei*: echinocandin, voriconazole, amphotericin-B/lipid amphotericin-B.

*C. glabrata*: echinocandin, amphotericin-B/lipid amphotericin-B.

**Adjust therapy according to clinical response and final identification of the Candida species.**

**Management of venous catheters:** see figure 8.2

**DURATION OF THERAPY:**

Uncomplicated: 7-10 days (minimum of 5 days after resolution of signs and symptoms of candidiasis).

Complicated: presence of source (septic thrombophlebitis) or organ infection (endophthalmitis, other organ, septic embolization /deep organ infection).

Treat for 14 days after resolution of all signs and symptoms of candidiasis.

\* May also use the Candida bedside scoring system.<sup>304</sup>

\*\* PNA Fish: Peptide nucleic acid fluorescence in situ hybridization.

**Figure 8-11** Management summary of patients at risk of invasive candidiasis.

**Table 8-3** Resistance of candida species to antifungal agents

<b>Candida species</b>	<b>Pattern of susceptibility/resistance to antifungal agents</b>			
	<b>Azoles</b>	<b>Echinocandins</b>	<b>Amphotericin B</b>	<b>Flucytosine</b>
<b><i>Albicans</i></b>	<2% resistance, usually in immunosuppressed patients on chronic fluconazole prophylaxis	Susceptible	Susceptible	Susceptible
<b><i>Tropicalis</i></b>	Susceptible	Susceptible	Susceptible	Susceptible
<b><i>Krusei</i></b>	Intrinsically resistant to fluconazole due to an altered cytochrome P450 isoenzyme. Susceptible to voriconazole, posaconazole	Susceptible	Decreased susceptibility may be seen	Decreased susceptibility may be seen
<b><i>Glabrata</i></b>	Mostly resistant to fluconazole due to changes in drug efflux. May be overcome with higher doses. Cross-resistance among azoles common	Susceptible	Susceptible but delayed in vitro killing kinetics	Susceptible
<b><i>Parapsilosis</i></b>	Susceptible. Emerging resistance to fluconazole noted	Mostly susceptible but higher MIC than other <i>Candida</i> spp.	Susceptible	Susceptible
<b><i>Dubliniensis</i></b>	Susceptible. Acquired resistance to fluconazole possible	Susceptible	Susceptible	Susceptible
<b><i>Lusitaniae</i></b>	Susceptible	Susceptible	Mostly susceptible	Susceptible
<b><i>Kefyr</i></b>	Susceptible	Susceptible	Susceptible	Susceptible
<b><i>Pelliculosa</i></b>	Mostly resistant to azoles	Susceptible	Susceptible	Susceptible
<b><i>Rugosa</i></b>	Mostly resistant to fluconazole. Mostly susceptible to voriconazole	NA	Mostly resistant	Mostly resistant

Susceptible denotes >90% susceptible strains; Mostly susceptible denotes 60–90% susceptible strains; Mostly resistant denotes <30% susceptible strains; NA: data not available.  
 Primary resistance of *C. lusitaniae* to amphotericin B rare among bloodstream isolates.  
 Organisms considered susceptible (using CLSI methods) when MIC is ≤8 µg/ml for fluconazole; ≤1 µg/ml for voriconazole; ≤1 µg/ml for flucytosine; ≤1 µg/ml for amphotericin B and ≤2 µg/ml for the echinocandins (anidulafungin, caspofungin, micafungin).

and AmB-deoxycholate (D-AmB) (0.3–1.2 mg/kg/day) for therapy of candidemia<sup>374-378</sup> showed that fluconazole was as effective as and better tolerated than D-AmB. The combination of fluconazole with D-AmB resulted in faster microbiologic clearance compared to fluconazole alone.<sup>260</sup>

Itraconazole therapy of invasive candidiasis has not been adequately evaluated. Oral itraconazole was comparable to oral fluconazole in a small study of pediatric patients with candidemia.<sup>379</sup>

Voriconazole was non-inferior to D-AmB followed by fluconazole for treating candidemia in non-neutropenic patients, and was better tolerated. Voriconazole is available orally and IV but is associated with drug interactions.<sup>380</sup>

Posaconazole possesses excellent in vitro activity against most *Candida* species but lacks an IV formulation.<sup>381</sup> Clinical data on primary treatment of invasive candidiasis are limited.

Polyenes Amphotericin B and its better tolerated lipid formulations have a broad activity against most *Candida* species.<sup>382-385</sup> Three lipid AmB products are available: AmB colloidal dispersion (ABCD) (Amphocil®), AmB lipid complex (ABLC) (Abelcet®), and liposomal AmB (L-AmB) (AmBisome®). Several studies have evaluated the lipid AmB preparations for the therapy of invasive candidiasis. A large randomized trial showed that ABLC (5 mg/kg/day) was as efficacious as and less nephrotoxic than D-AmB (0.7–1 mg/kg/day).<sup>386</sup> Data regarding treatment of invasive candidiasis with ABCD are limited to

**Table 8-4** Daily dosage of currently available antifungal agents for prophylaxis and therapy of hematogenous candidiasis

	Prophylaxis	Treatment
Fluconazole	200–400 mg PO	400–800 mg IV then PO
Itraconazole	200 mg IV or PO after loading	Not tested
Voriconazole	3–4 mg/kg PO bid after loading	4 mg/kg, IV/PO q12h after loading
AmB	0.5–07 mg/kg IV	1–1.5 mg/kg IV
ABCD (Amphotec®)	Not tested	1–4 mg/kg IV
ABLC (Abelcet®)	Not tested	1–5 mg/kg IV
L-AmB (AmBisome®)	1 mg/kg IV	1–3 mg/kg IV
Anidulafungin	Not tested	100 mg after 200 mg loading dose, IV
Caspofungin	Not tested	50 mg after 70 mg loading dose, IV
Micafungin	50 mg IV	100 mg, IV
IV, intravenous; PO, oral.		
Itraconazole		
Loading: 200 mg IV bid × 4 doses or 200 mg PO TID × 3 days. Use the IV formulation if severe infection, altered gut function or achlorhydria occurs. Capsule is better absorbed with fat- and protein-rich meals, whereas the solution should be taken 1 hour away from meals. Avoid capsule in immunosuppressed patients.		
Voriconazole: 6 mg/kg IV/PO q12 h × 2 doses.		
AmB, amphotericin B; ABCD, AmB colloidal dispersion; ABLC, AmB lipid complex; L-AmB, liposomal AMB.		

salvage trials<sup>387</sup> while the efficacy of L-AMB has been established in neonatal candidiasis (with response rates ranging from 72% to 100%)<sup>388–390</sup> and in a randomized controlled trial (RCT) in adults.<sup>391</sup> Overall, the three lipid AmB formulations are associated with less renal toxicity than D-AmB and among these, L-AmB (AmBisome) is the least nephrotoxic and results in significantly fewer infusion-related reactions.<sup>392,393</sup>

**Echinocandins** Anidulafungin, caspofungin and micafungin are broad-spectrum antifungal agents with minimal adverse events. They have somewhat comparable structure, pharmacology, in vitro and experimental activity and efficacy in randomized trials of invasive candidiasis.<sup>391,394–396</sup> Caspofungin (70 mg loading dose followed by 50 mg/day IV) was as effective as D-AmB but was better tolerated.<sup>394</sup> Micafungin (100 mg/day) was non-inferior to L-AmB (3 mg/kg/day)<sup>391</sup> while anidulafungin (200 mg loading dose, followed by 100 mg/day IV) was superior to fluconazole (400 mg/day).<sup>395</sup> Finally, two doses of micafungin (100 mg and 150 mg/day) were non-inferior to standard-dose caspofungin (50 mg/day) in a study of almost 600 patients. The higher micafungin dose did not provide additional benefit and all three regimens were extremely well tolerated.<sup>397</sup>

A pediatric multicenter RCT compared the efficacy of micafungin (2 mg/kg/day) with L-AmB (3 mg/kg/day) as first-line therapy in 98 pediatric patients with invasive candidiasis (19 premature infants, 57 younger than 2 years and 41 between 2 and 15 years old).<sup>398</sup> Treatment success was similar with both study drugs.

Of note, the minimum inhibitory concentration (MIC) of *C. parapsilosis* to echinocandins is 5–10-fold higher than that of other *Candida* species although the clinical significance of this finding remains unclear.

### Central venous catheter management (Fig. 8-12)

Removal of all CVCs in all patients with candidemia has been a standard textbook recommendation on the basis that CVC retention worsens outcome. However, and as mentioned earlier, most recent studies do not support this strategy.<sup>272</sup> We recommend CVC removal when venous access is no longer needed, or when replacement is easy and safe (non-tunneled, non-implanted CVC). In addition, CVCs should be removed in presence of high-grade fungemia (suggesting septic thrombophlebitis or endocarditis), otherwise unexplained hemodynamic instability, candidal organ infection (endophthalmitis, other) or pocket site infection, or unremitting and unexplained fever despite 72 hours of adequate antifungal treatment (appropriate dose and type for the infecting strain). Removal of CVC in *C. parapsilosis* candidemia is also recommended unless other sources such as contaminated TPN are responsible for the infection. In patients with candidemia and implantable or semi-implantable CVCs, medical antifungal treatment without CVC removal should be considered first, in the absence of the above-mentioned factors. The presence of severe neutropenia and mucositis (acute leukemia, BMT, other) suggests that the gut is the source of organisms and removal of CVC is unlikely to be beneficial in such setting.

Duration of therapy depends on whether candidemia is uncomplicated (in which case 1 week of therapy is appropriate) or associated with significant complications. Candidemia is considered complicated when a deep source of infection is present (septic thrombophlebitis of central veins, abdominal collection, other) or when seeding to deep organs is detected (endophthalmitis, other deep organ infection or multiple organs



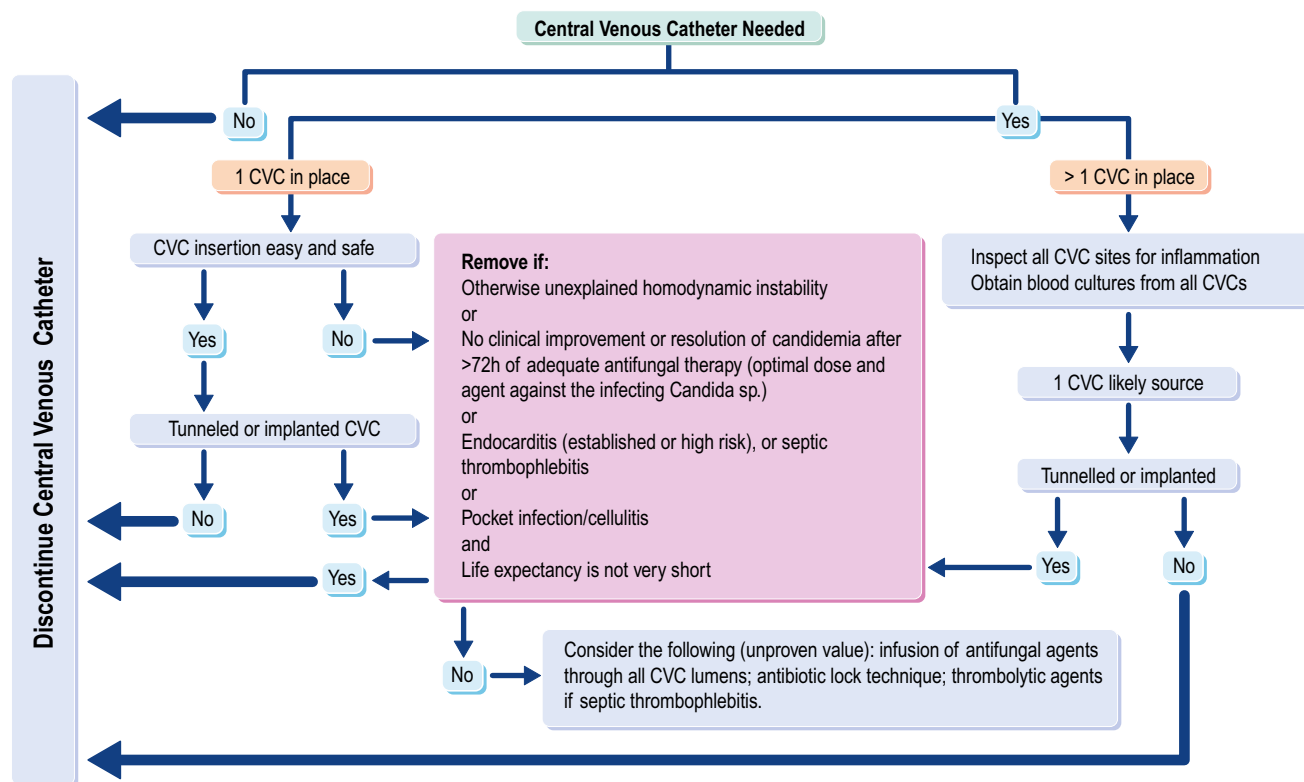
**Table 8-5** Practical considerations for selecting antifungal agents in patients with invasive candidiasis

<b>Host factors</b>
Avoid oral agents if:
High-risk population:
• Hematologic cancer, hematopoietic stem cell transplantation
• Severe immunosuppression
Hemodynamic instability
Gut dysfunction
Non-compliance
Avoid azoles if:
Prolonged and recent exposure to azoles
Liver dysfunction, significant
Drug–drug interaction, significant (fluconazole least affected)
Avoid amphotericin B products if significant renal dysfunction
<b>Pathogen</b>
Known in vitro antifungal susceptibility pattern: select agent likely to be effective against pathogen
<b>Site of infection (based on known tissue distribution of antifungal agent)</b>
Ocular or central nervous system infection:
• Avoid echinocandins
• If planning on using an amphotericin B product: select liposomal amphotericin B
• If planning on using an azole: select fluconazole or voriconazole
Urinary tract infection (pyelonephritis, cystitis, other): select fluconazole or 5-flucytosine. Avoid echinocandins (poor genitourinary penetration)

such as in ADC). Complicated candidemia requires treatment for  $\approx 14$  days after resolution of all signs and symptoms of candidiasis. In addition, removal of the source (drainage of an abscess, removal of a CVC in patients with septic thrombophlebitis) should be done.

#### Management summary for hematogenous candidiasis

- Consider empiric antifungal therapy in the ICU patient but only in presence of multifocal *Candida* colonization in a patient at high risk for invasive candidiasis who has unexplained fever that is refractory to broad-spectrum antibiotics (see Fig. 8-11).
- Obtain daily blood cultures until candidemia has resolved.
- When selecting the antifungal agent, carefully consider host factors, the infecting pathogen and the site of candidal infection (see Tables 8-3, 8-5).
- Use fluconazole in a non-neutropenic and hemodynamically stable patient, without evidence of endocarditis and no recent exposure to antifungal azoles; also, the local hospital epidemiology suggests unlikely infection with *C. krusei* or *C. glabrata*.
- Use fluconazole as oral “step-down therapy” for patients who have improved following other initial antifungal therapy and who were infected with susceptible organisms (*C. albicans*, *C. tropicalis* and *C. parapsilosis*).
- Consider voriconazole as oral “step-down therapy” for patients who have improved following other initial antifungal therapy and who were infected with a potentially fluconazole-resistant isolates (*C. krusei* or *C. glabrata*).
- Use an echinocandin or an AmB product for all other settings.
  - Because current data indicate comparable efficacy and safety profiles for the three commercially available echinocandins, we recommend using the least expensive agent.
  - If an AmB preparation is chosen, AmB-deoxycholate (D-AmB) is a reasonable option in patients at low risk for renal dysfunction while a lipid formulation (lipid AmB) is most appropriate when such dysfunction is present or likely to develop.
- Monitor patients with *C. parapsilosis* infection treated with an echinocandin (less susceptible) and patients with *C. glabrata* receiving voriconazole (cross-resistance between azoles can occur).<sup>255</sup>
- Consider removal of CVC if the appropriate conditions apply (see Fig. 8-12).
- If candidemia is complicated, treat for  $\approx 14$  days after resolution of all signs and symptoms of infection. Uncomplicated candidemia is effectively treated with a 7–10 day course of antifungal agents.
- Consider testing the susceptibility of *Candida* species to antifungal agents in certain settings<sup>399</sup> such as failure to respond to antifungal agents. Failure to respond to therapy may be microbiologic (drug resistance) or more commonly clinical (severe immunosuppression, presence of focus of infection). Remove all potential foci of infection.
- Consider the unique dosing attributes when treating pediatric patients.
  - D-AmB: children can generally tolerate higher D-AmB doses than adults.
  - Triazoles: higher doses of triazoles are required in children due to shorter half-life. For fluconazole, use 6–12 mg/kg/day; for voriconazole, increase the loading dose to 7 mg/kg/dose twice a day for 1 day, followed by 7 mg/kg/dose twice daily.
  - Echinocandins: micafungin dosing depends on age and ranges from 4 to 12 mg/kg/day because children require higher doses of micafungin and neonates require even higher doses than adults. The pediatric dose of anidulafungin is 0.7–1.5 mg/kg/day with no apparent difference in dosing between children and adults. For caspofungin, the loading dose is 50 mg/m<sup>2</sup> followed by 50 mg/m<sup>2</sup> daily (decrease to 35 mg/m<sup>2</sup> daily for hepatic insufficiency).



**Figure 8-12** Proposed management of CVC in non-neutropenic patients with candidemia. (Reproduced with permission from Nucci M, Anaissie E. Should vascular catheters be removed from all patients with candidemia? An evidence-based review. Clin Infect Dis 34(5):591, 2002.)

## Chronic disseminated candidiasis (CDC)

Treatment of CDC with AmB ( $\pm$  flucytosine) is typically associated with a 54% survival rate<sup>400</sup> although responses as high as 82% have been reported.<sup>401</sup> Patients failing to respond to or to tolerate D-AmB may be successfully treated with fluconazole (80–100% response),<sup>328,402</sup> or the lipid-AmB ABLC,<sup>403,404</sup> and L-AmB.<sup>405,406</sup> Variable outcomes have been reported with the few patients treated with caspofungin.<sup>407,408</sup>

We recommend a brief course of D-AmB (0.5–1 g) or lipid AmB (2–4 g) followed by fluconazole which should be continued for 1–2 months after complete resolution of all clinical signs of infection (radiologic scars may persist for years).

## Resistance to antifungal agents: clinical versus microbiologic

*Clinical resistance* refers to failure of therapy or rapid relapse of infection with a pathogen that did not demonstrate in vitro resistance to the therapeutic agent used. Host variables associated with clinical resistance include impaired immunity, undrained focus of infection (abscess, prosthetic material) and poor compliance. Low drug exposure is the most important drug-related variable responsible for clinical resistance and results from a suboptimal dose schedule, decreased absorption of oral agent because of gut dysfunction (nausea, vomiting, diarrhea, gut GVHD, mucositis, and others), drug–food interaction (alkaline gastric content for itraconazole capsules), poor oral intake (itraconazole capsule, posaconazole), and

drug–drug interaction with azoles and several drugs that result in decreased antifungal drug exposure).

*In vitro resistance* refers to the high MIC of the therapeutic agent against the offending pathogen (serum/tissue drug level is lower than the MIC of the antifungal agent against the pathogen) and can be primary (pathogen intrinsically resistant to the antifungal agent, no prior host exposure to the drug) or secondary (development of resistance following exposure to the antifungal agent). In vitro resistance can also be dose dependent (i.e., may be clinically overcome by higher doses of the antifungal agent) or inoculum size dependent (i.e., in vitro effect related to increasing the inoculum size of the tested fungus). However, strong correlation between in vitro susceptibility testing and clinical outcome of invasive candidiasis is lacking.

### Emerging resistance following prolonged drug exposure

The widespread use of newer antifungal agents in the immunocompromised population has been associated with emergence of drug resistance among patients exposed to such agents for prolonged periods of time.

**Azole resistance among *Candida* species** An association between prolonged use of an azole antifungal agent and the development of fungal infections with pathogens that are resistant to multiple azoles has been described among immunocompromised patients. Among patients with advanced HIV infection receiving oral itraconazole prophylaxis (mean duration, 14 months) or placebo, *C. albicans* isolates recovered from the last documented infection had significantly higher MICs for both itraconazole and fluconazole among

the itraconazole-treated patients compared with placebo patients.<sup>409</sup> Azole cross-resistance among clinical isolates of *C. albicans* has also been demonstrated in HIV-infected patients with oropharyngeal candidiasis.<sup>410,411</sup> Azole cross-resistance has also been reported in cancer patients who developed breakthrough fungemia caused by azole-resistant *C. tropicalis* after receipt of prophylactic itraconazole (35–105 days) followed by empirical fluconazole<sup>412</sup> and fluconazole-resistant *C. albicans* in BMT patients on chronic fluconazole prophylaxis.<sup>413</sup>

Azole cross-resistance among *Candida* species has also been investigated in vitro. A study of 429 clinical isolates from 45 Spanish centers reported proportional increases in the MICs of itraconazole, voriconazole, and fluconazole with a higher rate of cross-resistance among *C. tropicalis* than *C. albicans*, *C. glabrata*, *C. krusei*, or *C. parapsilosis*.<sup>414</sup>

Among 37 *Candida* isolates (41% were *C. glabrata*), a 20% rate of azole cross-resistance was noted.<sup>415</sup> Reassuring data, however, emerged from two large fungal surveillance studies which showed minimal changes in the in vitro susceptibility of *Candida* species to azoles. In one study (1997–2003), *C. albicans*, *C. parapsilosis*, and *C. tropicalis* were still highly susceptible to voriconazole, ravuconazole, and fluconazole ( $\leq 1.3\%$  resistance) with  $<20\%$  azole resistance among strains of *C. glabrata*.<sup>416</sup>

In another study of 90,000 isolates collected from 40 countries (1997–2005), the incidence of *C. albicans* resistance was extremely low ( $<1.5\%$ ).<sup>417</sup>

**Echinocandin resistance** The echinocandins possess excellent in vitro fungicidal activity against *Candida* species, including those resistant to azoles. However, resistance to the echinocandins has now been described in *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. krusei*, though it remains very rare.<sup>418–421</sup>

For a few isolates, cross-resistance to AmB or to azoles has been demonstrated.<sup>418,419</sup> Almost all isolates are cross-resistant to the other echinocandins.

## Cytokine therapy for hematogenous candidiasis

Protective immunity to invasive candidiasis is classically ascribed to innate immunity (particularly granulocytes) although recent data indicate that host defense against candidiasis is based on a complex interplay between innate and cell-mediated immunity.<sup>422</sup> Because the mortality associated with invasive candidiasis remains high despite recent availability of more potent antifungal agents, various cytokines have been evaluated as adjuvant therapy for invasive candidiasis, including granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage CSF (GM-CSF), macrophage-CSF (M-CSF) and interferon- $\gamma$  (IFN- $\gamma$ ). However, the clinical experience with these cytokines for the treatment of invasive candidiasis remains limited.<sup>423</sup>

## Prevention of hematogenous candidiasis

Prevention of severe candidal infections should focus on strategies that are effective and safe, and not associated with development of antifungal resistance. The best strategy relies on identifying the target population at highest risk for invasive

candidiasis (see Fig. 8-11), implementing simple infection control measures and antifungal chemoprophylaxis.

## Infection control measures

Transmission of *Candida* organisms from staff to patients and from patient to patient has been documented in several studies.<sup>85,86</sup> Strict handwashing is simple and may prevent nosocomial acquisition of organisms by patients. The use of artificial fingernails should be discouraged in areas housing high-risk patients because artificial fingernails may harbor yeasts.<sup>424</sup>

Cleaning, sterilization and disinfection of medical equipment, care of intravascular devices and sterile preparation of TPN infusates should follow standard guidelines.

## Antifungal chemoprophylaxis

Chemoprophylaxis with systemic antifungal agents should be reserved to the following high-risk patient populations.

### Hematologic malignancy and bone marrow/stem cell transplant patients

Patients with acute leukemia and/or BMT/HSCT recipients should be given antifungal chemoprophylaxis during the periods of risk. Several RCTs have shown that fluconazole prophylaxis (400 mg/day) reduces invasive candidiasis.<sup>245,246,425</sup>

Among HSCT recipients, fluconazole prophylaxis is continued for 75–100 days after HSCT.

Itraconazole capsules (200 mg/day) and solution (5 mg/kg/day) also reduce hematogenous candidiasis in patients with severe and prolonged neutropenia.<sup>426,427</sup>

Micafungin 50 mg/day was compared in a RCT to fluconazole 400 mg/day as antifungal prophylaxis in 882 neutropenic HSCT patients.<sup>428</sup> Overall, micafungin was as effective as fluconazole at reducing invasive candidiasis with a trend favoring micafungin protection against aspergillosis.

Posaconazole (200 mg given 3 times/day) was compared with fluconazole (400 mg/day) for prophylaxis in HSCT recipients with GvHD in one trial and with either fluconazole (400 mg/day) or itraconazole (200 mg twice/day) in neutropenic patients with acute leukemia in another. In both studies, breakthrough candidal infections were comparable in study groups though aspergillosis was decreased among posaconazole recipients.<sup>429,430</sup>

D-AmB is not an optimal prophylactic agent because of toxicity.<sup>431</sup> Among the lipid AmB formulations, L-AmB (AmBisome) is the least toxic<sup>392</sup> and may therefore be used as prophylaxis when no other alternatives are available. L-AmB has been used in doses of 1 mg/kg/day to 2 mg/kg/day thrice weekly.<sup>432,433</sup>

Caspofungin prophylaxis was compared with itraconazole in 200 patients with acute leukemia with comparable protection against candidiasis.<sup>434</sup>

Patients who develop CDC may need to undergo further cytotoxic chemotherapy or HSCT/BMT. Two reports suggest that secondary prophylaxis during subsequent periods of neutropenia is successful in  $>80\%$  of patients.<sup>435,436</sup>

### High-risk ICU patients

A recent metaanalysis identified four double-blind RCTs (626 patients) that compared fluconazole to placebo for prevention of fungal infections in the ICU. Fluconazole prophylaxis



significantly reduced the incidence of fungal infections but was not associated with a statistically significant survival advantage.<sup>437</sup>

### Solid organ transplantation

The incidence of invasive fungal infections following SOT ranges widely depending on patient selection, the organ transplanted and whether antifungal prophylaxis was used. Among SOT patients, the recipients of liver, small bowel and pancreatic transplants are at higher risk for invasive candidiasis.

A metaanalysis of 14 randomized trials with 1497 participants evaluated the efficacy of antifungal prophylaxis in SOT recipients.<sup>438</sup> The following risk factors for invasive fungal infections were identified: renal or hepatic dysfunction; known fungal colonization pretransplantation; prior broad-spectrum antimicrobial use; large blood transfusion requirement; prolonged ICU length of stay and posttransplant surgery, including laparotomy and retransplantation. Overall, antifungal prophylaxis did not reduce mortality in SOT recipients. However, prophylaxis with fluconazole significantly reduced invasive candidiasis in liver transplant recipients and a similar though less robust effect was observed with itraconazole and L-AmB.

**Liver transplantation** Risk factors for fungal infections following liver transplantation include repeated operation, higher intraoperative transfusion requirements, longer operation time and renal failure. In a double-blind, placebo-controlled RCT, fluconazole (400 mg/day for 10 weeks after transplantation) significantly reduced invasive fungal infections.<sup>439</sup> Because of these and the metaanalysis findings, fluconazole prophylaxis is recommended for 4 weeks following liver transplantation.

L-AmB has been evaluated in five RCTs in liver transplant recipients and appears to be protective against candidiasis although effective prophylaxis against aspergillosis required a dose of 5 mg/kg/day.<sup>440</sup>

A more recent study employed L-AmB prophylaxis (1 mg/kg/day for 7–10 days) in 22 high-risk liver transplant recipients. One-year survival was 80% and a low rate of severe fungal infections was seen (two aspergillosis but no *Candida* infection).<sup>441</sup>

**Pancreatic transplantation** Risk factors for fungal infections among these patients include receipt of more than one organ (pancreas and kidney) from an older living donor, enteric instead of a bladder drainage procedure, preoperative peritoneal dialysis, pancreatitis after reperfusion and pancreatic retransplantation. Fluconazole prophylaxis is also recommended for 4 weeks after transplantation.

**Bowel transplantation** Risk factors for invasive candidiasis include repeated operation, higher intraoperative transfusion requirements, longer operation time, renal failure, intraoperative contamination and ischemia. Fluconazole prophylaxis is recommended for 4 weeks after transplantation.

**Lung transplantation** The incidence of invasive candidiasis is relatively low in lung transplant recipients, in whom mould infections are a particular problem. Itraconazole prophylaxis used to prevent mould infections also prevents invasive candidiasis.<sup>442</sup>

**Other organ transplantation** Controlled trials of antifungal prophylaxis have not been conducted among renal and cardiac transplant recipients. It is likely that fluconazole will be similarly effective at preventing invasive candidiasis in these patient populations.

### Preterm neonates

In a large, multicenter retrospective study, Italian investigators reviewed the records of 465 neonates who weighed <1500 g at birth and were admitted to the NICU during 1998–2003. Patients born between 1998 and 2000 did not receive fluconazole prophylaxis (group A, n = 240) and were compared with those born between 2001 and 2003 and treated with fluconazole until the 30th day of life (45th for neonates <1000 g at birth; group B, n = 225). Weekly surveillance cultures were obtained from all patients. Fluconazole-treated patients (group B) had significantly lower fungal colonization and systemic fungal infections (10/225; 4.4% vs 40/240; 16.7% in group A). These benefits were particularly striking among neonates weighing <1000 g at birth although still significant for those weighing 1001–1500 g at birth. In addition, the rate of progression from colonization to infection was also significantly lower in group B. Any cause mortality rate was similar in the two groups, but in colonized infants (n = 159), it was significantly lower in group B (3.7% vs 18.1%). Fluconazole was well tolerated and no antifungal resistance was observed.<sup>443</sup>

A double-blind RCT enrolled 100 preterm infants with birth weights <1000 g to receive IV fluconazole (50 patients) or placebo (50 patients) for 6 weeks. Weekly surveillance cultures were obtained from all patients. During the 6-week treatment period, fungal colonization was significantly higher in the placebo group (30/50, 60% vs 11/50, 22%). Similarly, significantly higher rates of invasive fungal infection were seen in the placebo group (10/50, 20% vs none in the fluconazole group).<sup>444</sup>

The same investigators subsequently conducted a double-blind RCT comparing their previous dosing schedule (group A, 41 patients) to a less frequent dosing schedule of twice a week (group B, 40 patients) of fluconazole prophylaxis for up to 6 weeks in preterm infants weighing <1000 g at birth and with an endotracheal tube and/or CVC. Weekly surveillance cultures were obtained on study patients. No significant differences in *Candida* colonization and invasive infection were observed, leading the investigators to conclude that twice-weekly dosing of prophylactic fluconazole should be considered in this patient population.<sup>445</sup>

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# Cryptococcus

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*Cryptococcus neoformans* is an encapsulated yeast species that causes infections in animals and in humans in almost all areas of the world. This fungus can infect apparently normal hosts but much more frequently and with greater severity it causes disease in immunocompromised individuals. The disease may vary from localized to disseminated and from acute to chronic. The infection usually initiates in the lung, following inhalation of the fungal cells, and spreads hematogenously to the brain, causing life-threatening meningitis or meningoencephalitis.

Cryptococcosis, an uncommon disease before the acquired immune deficiency syndrome (AIDS) epidemic, has emerged as an important cause of illness and death in human immunodeficiency virus (HIV)-infected people.

The first case of cryptococcosis was reported in 1894 by Busse who named the fungus *Saccharomyces hominis*.<sup>1</sup> In the same year, but separately, Sanfelice cultured the yeast from peach juice. He demonstrated its pathogenicity in experimental animals and named the fungus *Saccharomyces neoformans* because of its tendency to form tumor-like lesions in tissues.<sup>2,3</sup> In 1901 Vuillemin renamed the fungus *Cryptococcus hominis* because of the lack of ascoconidia production typical of *Saccharomyces* spp.<sup>4</sup> Many other names were coined to refer to this fungus, such as *Saccharomyces tumefaciens*, *Torula histolytica*, *Debaryomyces hominis*, *Cryptococcus histolyticus*, and to related infections, such as European blastomycosis and torulosis, causing considerable confusion.

In 1950, however, the taxonomic relationship of Sanfelice's organism to other yeasts was clarified and the terms *C. neoformans* and cryptococcosis were universally adopted. Subsequently, on the basis of antigenic differences in the capsular polysaccharide, five serotypes (A, B, C, D and AD) were identified which were grouped in two varieties: var. *neoformans* (serotype A, D and AD) and var. *gattii* (serotype B and C) that were shown to correspond to two morphologically distinct teleomorphs, *Filobasidiella neoformans* and *F. bacillispora*, respectively.<sup>5,6</sup> Given their significant divergence at molecular level, the two varieties of *C. neoformans* have been recently awarded separate species status, as *C. gattii* and *C. neoformans*; in addition, distinction of *C. neoformans* in two varieties, var. *grubii* (serotype A) and var. *neoformans* (serotype D), has been proposed.<sup>7-9</sup>

In the last few years, the availability of the complete genome sequence of *C. neoformans* has greatly contributed to the knowledge of this yeast.<sup>10</sup>

The history of this fungus and the disease that it caused was reviewed in depth by Drouhet.<sup>11</sup> Progress in the understanding of this fungal pathogen was summarized in two exhaustive monographs, one edited in the 1950s and the other at the end of the 1990s.<sup>12,13</sup>

## Mycology

The genus *Cryptococcus* includes yeasts, oval to round in shape, that reproduce by multilateral budding. These yeasts are non-fermentative and are characterized by their ability to assimilate inositol as a sole carbon source, to produce urease, and to react with diazonium blue B.<sup>14</sup>

*Cryptococcus* is a polyphyletic genus that includes over 50 species. Molecular studies have demonstrated that some species that include isolates with some variable physiologic and/or biochemical characteristics are taxonomically heterogeneous.

*C. neoformans* and *C. gattii* are considered the only *Cryptococcus* species pathogenic for humans, due to their unique ability to grow at 37°C. However, other species, such as *C. albidus*, *C. laurentii* and *C. curvatus*, have occasionally been reported as causes of infection.<sup>15-17</sup> In addition, *C. adeliensis*, a new species isolated from algae in Antarctica that can be misidentified as *C. albidus*, has been reported as a cause of meningitis in a patient with acute myeloid leukemia.<sup>18</sup> It should be remembered, however, that the significance of the isolations of these rare species from non-sterile sites remains doubtful in the absence of both culture and histologic evidence of tissue invasion.

*C. neoformans* and *C. gattii* are heterothallic encapsulated yeasts that produce a basidiomycetous teleomorph under experimental conditions. During the haploid phase of their life cycle these yeasts grow on routine culture media, developing, in 48–72 hours, white to cream colored colonies that are more or less mucoid according to the capsule size of the cells. Microscopically, unicellular cells of the fungus are spherical to oval in form and variable in size, with single or multiple buds. *C. gattii* cells may exhibit some elongated cells with greater size.

The individual cells are surrounded by a polysaccharide capsule the size of which depends on the genetics of the strain and on the conditions of growth: nutritional factors, CO<sub>2</sub> tension and temperature. The capsule may be visualized by mounting the cells in an India ink preparation. It appears as a clear halo around the yeast in a black field, as the ink carbon particles do not penetrate the capsule. The diameter of the cell can vary from 2–5 µm in capsule-deficient or poorly encapsulated strains to 30–80 µm in heavily encapsulated cells. In nature and in culture media most of the strains are poorly capsulated, whereas in tissues they usually show a large capsule. The capsule is mainly composed of polysaccharides, namely glucuronoxylomannan, which represents approximately 90%, galactoxylomannan and mannoprotein. With scanning electron microscopy, the capsule appears to be composed of a loose radiated network of microfibrils attached to the cell wall.<sup>19</sup> Differences in the glucuronoxylomannan structure depend on the degree of mannosyl substitution and the molar ratios of mannose, xylose and glucuronic acid.<sup>20</sup> These differences provide the basis for the separation of strains into serotypes, known as A, B, C, D and AD.<sup>21–23</sup> Methods for serotype identification have been developed using polyclonal absorbed and monoclonal antibodies in slide agglutination and immunofluorescence tests.<sup>23,24</sup>

The cell wall defines the shape and protects the cell against osmotic stress and is clearly visible with light microscopy as a highly refractive double-contoured structure. Glucose is the main component of the cell wall, which also contains hexosamine, nitrogen and phosphate. These components are arranged in multiple parallel fibrillar layers of sheath-like plates, as observed with electron microscopy. The water-soluble

fraction of the cell wall contains a polysaccharide mainly composed of (1,6)-β-D glucopyranans and the water-insoluble component contains primarily (1,3)-α-D glucan. These compounds are respectively responsible for the relative resistance to pneumocandins<sup>25</sup> and for the low diagnostic yield of the (1,3)-β-D glucan measuring test.<sup>26</sup>

The cytoplasm shows typical eukaryotic cellular structures, namely a nucleous, mitochondria, an endoplasmic reticulum and ribosomes. In addition, several vacuoles can be seen with light microscopy and are presumed to contain storage lipids.

The yeast-like cell represents the anamorphic form of *C. neoformans* and *C. gattii*, which is found in both clinical and environmental samples. Several differences have been recognized between the two anamorphic species in relation to their life cycles, physiology, ecology, genetics and pathobiology. The teleomorphic or sexual state, which reproduces by basidioconidia, was identified in crossing experiments in vitro; that is, by crossing the two mating types, *a* and *α*, of *C. neoformans* or *C. gatti* compatible strains.<sup>5,6</sup> Under such conditions hyphae are produced that give rise to basidioconidia that differ in size and shape in the two species, *Filobasidiella neoformans* and *F. bacillispora*. *F. neoformans* produces chains of spherical basidioconidia, whereas *F. bacillispora* produces oval bacilli-form basidioconidia. Sexual reproduction between partners of both the same and opposite mating type may occur.<sup>27</sup> These teleomorphic states, however, have never been demonstrated in nature or in patients.

Many biochemical differences have been identified in the two species (Table 9-1). They differ in their ability to assimilate L-malic and fumaric acids, D-proline and D-tryptophan.<sup>28–30</sup> Both assimilate creatinine but differ in the regulation of the

**Table 9-1** Differences between *Cryptococcus neoformans* and *C. gattii*

	<i>C. neoformans</i>	<i>C. gattii</i>	References
Teleomorph	<i>Filobasidiella neoformans</i>	<i>Filobasidiella bacillispora</i>	5, 6, 241
Serotypes	A, D and AD	B and C	21–23
Growth at 37°C	+	+ weak	241
Assimilation			
L-malic acid	-/weak, in 5 days	+ in 2 days	28
Fumaric acid	-/weak, in 5 days	+ in 2 days	28
D-proline	-	+	29
D-tryptophan	-	+	30
Inhibition of creatinine assimilation by NH <sub>3</sub>	yes	no	31
Inhibition of urease activity by EDTA	no	yes	32
Colorimetric tests*			
color change of GCP agar	no	yes, red	242
color change of CGB agar	no	yes, blue	33
*Intermediate/false results rarely occur. GCP, glycine, cycloheximide (1.6 µg/ml), phenol red. CGB, canavanine, glycine, bromothymol blue. EDTA, ethylenediamine tetraacetic acid.			

creatinine metabolism. The synthesis of creatinine deiminase is repressed by ammonia in *C. neoformans*, but not in *C. gattii*.<sup>31</sup> Both species exhibit urease activity which, in *C. gattii*, is inhibited by EDTA.<sup>32</sup> Two agar media, canavanine glycine bromothymol blue (CGB) agar<sup>33</sup> and D-proline agar,<sup>29</sup> can be used to distinguish the two species. Intermediate results, however, may be produced with both media requiring confirmation by other tests.<sup>34</sup>

## Ecology

*C. neoformans* is found worldwide, invariably isolated from pigeon droppings and soil contaminated by avian excreta, as reported by several studies after Emmons' original studies.<sup>35</sup> In contrast, *C. gattii* is usually found in tropical and subtropical climates associated with *Eucalyptus* trees. Recently, however, this species has been isolated from several environmental samples in Vancouver Island, British Columbia mainland (Canada) and later on in Washington and Oregon states (USA) associated with human and animal infections.<sup>36,37</sup> A specific route of *C. gattii* introduction to the Pacific Northwest and the new environmental niches are at present under investigation.

The ability of *C. neoformans* to colonize pigeon excreta has been attributed to the ability of this species to utilize creatinine as a source of nitrogen.<sup>38</sup> Creatinine, however, is also utilized by *C. gattii* which, in contrast, is unable to survive in avian droppings. This results from the fact that in *C. neoformans* the synthesis of creatinine deiminase, which cleaves creatinine to ammonia and methylhydantoin, is repressed by the overproduction of ammonia, while in *C. gattii* there is no enzyme repression and the strong alkalization inhibits its growth.<sup>31</sup> In fresh or wet pigeon droppings the strong alkalization produced by bacterial decomposition also markedly reduces the number of *C. neoformans* cells which, in contrast, are highly resistant in dry excreta.<sup>39</sup> The yeast cells may remain viable for almost 2 years and possess a reduced capsule.<sup>40</sup> The small size of the yeasts (1–3 µm) is compatible with alveolar deposition. Avian excreta are more likely to be positive for *C. neoformans* in sheltered environmental locations than in those exposed to sunlight, due to the high susceptibility of this fungus to UV radiation. Despite the presence of *C. neoformans* in their crops, pigeons are resistant to cryptococcal disease because of the inhibitory effect of their elevated body temperature (41–43°C) and of their gut's bacterial biota.<sup>40</sup>

According to recent studies, the environmental habitat of *C. neoformans* appears to be related to trees and plant material as it is for *C. gattii*. Both species have a laccase enzyme with phenoxidase activity, which enables these fungi to degrade lignin, thus conferring on them the ability to use tree and plant material as their habitats.<sup>41,42</sup> Pigeons only contribute to the propagation of the fungus by providing an enriched medium for fungal growth and dispersing the fungus from their contaminated beaks and feet.<sup>40</sup>

The discovery in 1990 of a specific association of *C. gattii* serotype B with *Eucalyptus camaldulensis* trees in Australia's Barossa Valley provided an insight into the natural habitat of this yeast.<sup>43</sup> Since then, this serotype has been isolated from *Eucalyptus* species in Australia, Brazil, California, India,

Italy, Mexico and New Guinea, from other trees (*Guettarda* sp., *Cassia grandis*, *Erythrina velutina*) in Brazil,<sup>44</sup> and from firs and oaks in Vancouver Island.<sup>45</sup> The occurrence in Spain of five outbreaks of severe pulmonary cryptococcosis in goats caused by var. *gattii* serotype B indicates a wider distribution of this pathogen, possibly related to reforestation with *Eucalyptus* trees.<sup>46</sup> *C. gattii* serotype C has only recently been isolated from the environment, from almond trees (*Terminalia catappa*) in Colombia.<sup>47</sup> Also *C. neoformans* serotype A has been isolated from *Eucalyptus* trees and from several other genera of trees in Brazil and Colombia.<sup>48–50</sup> According to available epidemiologic data, the *neoformans* species seems not to be associated with a particular genus of trees but rather with a specialized niche resulting from the natural biodegradation of wood.<sup>51</sup>

The isolation of these cryptococcal species from highly contaminated environmental sites has been improved by the use of selective-differential media containing glucose, creatinine and *Guizotia abyssinica* (niger seeds) that enhance the growth of the yeast colonies and confer on them a brown color due to the production of melanin.<sup>52</sup> The addition of chloramphenicol and 0.1% diphenyl facilitates their growth by inhibiting the growth of contaminating bacteria and moulds.<sup>53,54</sup>

## Pathogenesis and pathology

The fungus–host interaction has stimulated a large number of studies, mostly carried out with *C. neoformans* var. *neoformans*, and they have greatly contributed to understanding the pathogenesis of cryptococcosis. Current knowledge concerning the dynamics of the fungus–host interaction has been presented in recent reviews.<sup>55</sup>

## Virulence factors

*Cryptococcus neoformans* has several means by which to escape the host's defenses, to survive and to multiply inside the host. Factors that contribute to the virulence of the fungus are mainly related to its ability to grow at 37°C, to produce a thick polysaccharide capsule and release soluble products into the bloodstream, and to synthesize melanin. Other virulence factors include urease, phospholipase, proteases and mannitol production. These virulence factors have a dual use: function in mammalian virulence and function in the environment.<sup>56</sup>

The ability to grow at 37°C is essential for survival in the human host. Except for *C. neoformans*, *C. gattii* and occasionally *C. laurentii* and *C. albidus* isolates, other species cannot grow well at 37°C and are considered to be non-pathogenic for humans. *C. gattii* as well as *C. neoformans* serotype D isolates appear to be more sensitive to high temperature (37–39°C) compared to *C. neoformans* serotype A isolates.<sup>57</sup> The ability to grow at 37°C was proven to be under genetic control. The calcineurin A gene, *CNCAL1*, was first identified that encodes a protein, a serine-threonine specific phosphatase activated by Ca<sup>2+</sup>-calmodulin, essential for survival at 37°C at pH 7.3–7.4 in an atmosphere of approximately 5% CO<sub>2</sub>.<sup>58</sup> Recently other genes have been identified which need to be intact for high temperature growth.<sup>59</sup>



The capsule, as well as the soluble polysaccharide released from the yeast cells during infection, plays a significant role in pathogenicity as it protects the yeast cells from phagocytosis and from cytokines induced by the phagocytic process, and suppresses both cellular and humoral immunity.<sup>60-62</sup> In soil and under laboratory conditions the capsule size is relatively small and the poorly capsulated yeasts are easily inhaled. In contrast, in the host the physiologic CO<sub>2</sub> concentration, the limitation of ferric iron and the presence of serum increase the capsule size, making the yeast resistant to host defenses.<sup>63,64</sup> All the components of the capsule have been recognized to elicit an antibody response, but only the mannoprotein component stimulates cell-mediated immunity, while glucuronoxylomannan and galactoxylomannan components are poorly immunogenic.<sup>65</sup> A large capsule can block the opsonic effect of complement and anticryptococcal antibodies, can limit production of nitric oxide (which is an inhibitor of cryptococcal cells) and can interfere with the antigen presentation process.<sup>66</sup> In addition, a large capsule enhances HIV infection and induces apoptosis, a possible factor in cerebral edema.<sup>13</sup> Capsular polysaccharide confers a high negative charge to the yeast cell's surface, causing electrostatic repulsion between the yeast cell and the negatively charged host effector cells.<sup>67</sup> As a facultative intracellular pathogen, the yeast releases the capsular polysaccharide intracellularly and its accumulation in cytoplasmic vesicles has a negative effect on the macrophage and allows the yeast to survive.<sup>68</sup> Several genes have been identified and all proven essential for capsule production and virulence.<sup>69-73</sup>

The ability of *C. neoformans* to produce melanin depends on a fungal phenoloxidase enzyme. This enzyme is bound to the cell membrane and catalyzes the reaction in the presence of phenolic compounds, including catecholamines. This ability was first observed in agar containing a *Guizotia abyssinica* seed extract<sup>52</sup> and subsequently in medium containing caffeic acid extracted from the seeds, and iron compounds.<sup>74</sup> The phenoloxidase enzyme has been identified as a laccase, encoded by two genes, *CNLAC1* and *CNLAC2*, but only *CNLAC1* is expressed significantly under most conditions and deletion of *CNLAC2* results in no reduction in virulence in mice.<sup>75,76</sup> Melanin is deposited in cell walls, conferring a brown color to the yeast cells; it promotes cell integrity and increases its negative charge, protecting them from phagocytosis.<sup>75</sup> Melanin prevents in vitro T cell response and cytokine secretion, and reduces antibody-mediated phagocytosis.<sup>67,75</sup> Other postulated functions include protection from oxidants and host oxidative killing, from temperature extremes, iron reduction, UV light, amphotericin B and microbicidal peptides.<sup>75,77</sup> Melanin is essential for extrapulmonary dissemination, facilitating the escape from the lung as it allows survival of the yeast inside the alveolar macrophages and their transport into the lymph nodes and subsequently into the bloodstream. Melanin, however, is not required for growth in the lung or in the brain.<sup>78</sup> Melanogenesis has been suggested to occur in vivo during infection, given the existence of melanin precursors such as L-dopa and epinephrine in tissue. The high level of catecholamines in the brain supports the speculative basis for the remarkable neurotropism of this pathogen.<sup>79</sup> However, the role of melanin production in virulence remains uncertain since the phenoloxidase activity of the fungus is severely reduced at 37°C.<sup>80</sup>

The production of major virulence determinants, namely the capsule and melanin, was shown to be linked to the expression of several genes (transcription factor Ste12, PAK kinase Ste20 and Ste3 pheromone receptor) contained in the *C. neoformans* MAT locus. Differences in virulence between *C. neoformans* opposite mating type cells have been investigated and  $\alpha$  cells were shown in animal models to be more pathogenic than  $\alpha$  cells.<sup>81,82</sup> However, no difference was reported by other authors who, nevertheless, found  $\alpha$  cells more efficient than  $\alpha$  cells in crossing the blood-brain barrier during coinfection.<sup>83,84</sup> The hypervirulent clone of *C. gattii* that caused the Vancouver Island epidemic was shown to descend from two  $\alpha$  mating type parents.<sup>85</sup>

It is of interest that some interactions between virulence factors and drugs may occur: cyclosporine-tacrolimus inhibit high-temperature growth,<sup>58</sup> indinavir inhibits capsule and urease production,<sup>86</sup> while melanin reduces susceptibility to amphotericin B and caspofungin.<sup>87</sup>

## Host defenses

From the environment, poorly or unencapsulated dried yeast cells are inhaled and can reach the alveolar spaces, where they gradually rehydrate and acquire their characteristic polysaccharide capsule. Development of the disease largely depends on the competence of the host's cellular defenses and the number and virulence of the inhaled yeast cells.

Protective immunity against *C. neoformans* is achieved by the integration of the innate and the adaptive (or antigen-specific) responses, as described in recent reviews.<sup>55,88,89</sup> Innate immunity acts early and is activated by specific recognition of molecular structures of *C. neoformans* and its secreted products by a set of cellular receptors that recognize conserved molecular structures shared by large groups of pathogens. These receptors, that include mannose receptors (MRs), CD14, CD18 and the Toll-like receptors, are expressed on natural killer (NK) cells and phagocytes, namely polymorphonuclear neutrophils, monocytes, macrophages and dendritic cells.

In the lung *C. neoformans* first interacts with alveolar macrophages and dendritic cells, that participate in fungal recognition, phagocytosis, antigen presentation and activation of the host response. Although alveolar macrophages possess "a machinery" for antigen presentation, their main contribution to antifungal defence is through phagocytosis and killing.<sup>90</sup> Macrophages can ingest the yeast but have limited efficacy in eliminating the fungus. However, macrophages are effective in producing proinflammatory monokines (IFN- $\alpha$ , IL-1 $\beta$ , IL-6) for the recruitment of neutrophils, monocytes, NK cells and T cells from the bloodstream into the lung. The recruited cells are effective in killing the yeasts by intracellular and extracellular mechanisms.<sup>91,92</sup> Intracellular killing occurs through lysosomal fusion, phagosomal acidification, sequestration of iron and enzymatic degradation of fungal proteins, while extracellular killing is mediated by antifungal peptides, nitric oxide and possibly reactive oxygen intermediates. The non-phagocytic effector cells also kill *C. neoformans* – NK cells mainly by release of perforin from their granules and CD8 T lymphocytes by a similar non-oxidative mechanism involving granulysin.<sup>60,93</sup>

Dendritic cells provide a link between innate and adaptive immunity as they are highly efficient in the antigen presentation to T cells. In their immature form they phagocytose

*C. neoformans* via MRs and Fc $\gamma$  receptor IIs (Fc $\gamma$ RIIs). As they mature, dendritic cells migrate to the T cell area of lymphoid organs where they communicate to T cells the nature of the antigen they are presenting, thus initiating the qualitatively different T helper (Th) immune response.<sup>94</sup> Entry of *C. neoformans* into dendritic cells through MRs (non-opsonic phagocytosis) is required to activate a protective Th1 cell response mediated by IL-12. CD4 T cells with Th1 profile activate the effector arm of cell-mediated immunity (CMI) to kill *C. neoformans*; the production of IFN- $\gamma$  cytokines with the help of opsonizing antibodies results in activation of phagocytosis at the site of the infection. In contrast, the entry into dendritic cells through Fc $\gamma$ RII (opsonic phagocytosis) results in the production of IL-4 and/or IL-10 and activation of non-protective Th2 responses/induction of regulatory T cells. The main function of Th-reg cells is counterregulation or suppression of immunoresponses mediated by Th1 and Th2 cells.

CMI response remains the critical component for protection against *C. neoformans*. Specific T cells act through direct cytotoxic effects on the fungus and produce IFN- $\gamma$  and other lymphokines that activate effector cells, such as phagocytes and NK cells.

The antibody response usually is poor and non-protective, but specific antibodies can opsonize the yeast cells, enhancing antibody-dependent cell-mediated cytotoxicity.

Soluble *C. neoformans* mannoprotein has the capacity to elicit delayed-type hypersensitivity and Th1-like cytokines, both critical to the clearance of this yeast.

The complement system, which is a non-specific humoral defense system, greatly contributes to the host's defenses against *C. neoformans* as it enhances the efficacy of anticryptococcal antibodies and provides opsonins for phagocytosis and chemotactic factors for the recruitment of inflammatory cells. The pathway of complement activation by *C. neoformans* depends on the state of encapsulation of the yeast cells and the presence of specific antibodies to fungal antigens. Encapsulated yeasts are potent activators of the alternative complement system with resultant deposition of C3 breakdown products on the capsular surface. Disseminated cryptococcosis may result in a complement-deficient state due to the consumption of complement factors.<sup>95</sup>

The host immune response to cryptococcal infection is the result of a complex interplay between cellular and humoral immunity. Impairment of the host's defenses against *C. neoformans* can lead to dissemination of the infection, most likely by migration of macrophages with ingested fungal cells from the lung to the draining lymph nodes and via the bloodstream to the brain. At the blood-brain barrier, astrocytes and microglial cells have been proven to be active in response to cytokine stimulation against *C. neoformans*, since astrocytes can produce nitric oxide and the microglial cells can ingest yeast cells when supplied with antibodies to glucuronoxylomannan.<sup>96,97</sup> But in the absence of cytokine stimulation both cells are ineffective in killing the fungus.<sup>98</sup>

## Epidemiology

The prevalence of cryptococcosis in a population appears to be a function of the number of immunocompromised individuals and the magnitude of exposure to this environmental

pathogen. The hypothesis of the acquisition of the infection from the environment is supported by molecular epidemiologic studies that showed concordance of clinical and environmental isolates.<sup>99,100</sup>

The possibility of transmission of cryptococcosis following transplantation of infected tissue is well known.<sup>101-103</sup> However, human-to-human natural transmission as nosocomial or mother-to-child infection has only recently been reported.<sup>104,105</sup>

## Route of infection

A respiratory route of infection is supported by evidence such as the size of the poorly encapsulated yeast cells in the environment (less than 3  $\mu$ m in diameter) that is compatible with alveolar deposition.<sup>106</sup> In addition, cryptococcal pneumonia has been recognized as a distinct clinical disease and patients with meningitis usually have a primary pulmonary lymph node complex, or carry healed pulmonary granulomas, or are affected with disseminated lung infections.<sup>107,108</sup> A gastrointestinal route has also been suggested by anecdotal reports. Traumatic inoculation of *C. neoformans* yeast into the skin has also been reported as a cause of primary cutaneous cryptococcosis.<sup>109,110</sup> The majority of cases of cutaneous cryptococcosis, however, reflect dissemination from internal sources.<sup>111,112</sup>

## Prevalence

*Cryptococcus neoformans* commonly occurs in urban areas but although human exposure to the fungus is probably a common event, cryptococcosis remains a sporadic disease.

There is no sensitive and specific skin test to determine the prevalence of subclinical infections in the human population. Only people repeatedly exposed to *C. neoformans*, such as pigeon breeders or laboratory mycologists, have shown a high percentage of positive skin test reactions.<sup>113</sup>

Until the first half of the 20th century cryptococcosis was rarely reported. A rising incidence of the infection was observed starting in 1965 when the availability of a new serologic test for the detection of cryptococcal polysaccharide in body fluids made diagnosis easier.<sup>114</sup> Improvements in mycologic diagnosis and greater awareness of this infection, combined with advances in medical procedures and prolonged survival of immunocompromised patients, contributed to the steady rise in the number of reports of this fungal disease. Before the AIDS outbreak, clinical experience was <1000 patients per year in the United States.<sup>115</sup>

A dramatic increase was observed with the advent of the AIDS pandemic and, since then, HIV infections account for more than 75% of the predisposing factors.<sup>116,117</sup> Consequently, diagnosis of cryptococcosis in patients with an unknown predisposition always suggests an evaluation for HIV infection. The risk for cryptococcosis occurs late in the course of HIV infection, usually when the CD4+ lymphocytes are less than 100/mm<sup>3</sup>.<sup>118</sup> Extrapulmonary cryptococcosis in HIV-infected individuals is defining of AIDS.

The prevalence of cryptococcosis among AIDS patients in the early period of the epidemic was 2–10% in Western Europe and the USA and more than 15% in Central Africa and Southeast Asia.<sup>116,119,120</sup> The high prevalence of cryptococcal infection in Burundi was related to increased exposure to the

fungus present in household dust in 50% of patients' homes.<sup>121</sup> During the 1990s, the prevalence of this mycosis progressively declined in developed countries, at first as a result of the widespread use of fluconazole and later due to successful treatment with new antiretroviral drugs (HAART). Population incidence in metropolitan areas of the USA declined from 4.9 to 0.2–0.9 cases/100,000 population.<sup>122</sup> Despite HAART, however, cryptococcosis continues to carry a significant morbidity and mortality in the resource-limited countries, such as Africa and Southeast Asia, that are now experiencing the greatest burden of global AIDS epidemic. In addition, in industrialized countries cryptococcosis continues to occur in those with undiagnosed HIV infection and in socio-economically disadvantaged HIV-infected people who do not have access to HAART.

*Cryptococcus neoformans* is the most common species affecting patients with AIDS even in geographic areas where *C. gattii* is present in the environment.<sup>123</sup> Indeed, in Australia nearly all cases of cryptococcosis associated with AIDS are caused by *C. neoformans*. The rarity of *C. gattii* infections in AIDS patients has been related to the rare exposure of HIV-infected people to the rural environmental niche of *C. gattii*.<sup>123</sup>

*Cryptococcus gattii* infections occur mainly in immunocompetent hosts in the rural areas of Australia, and in endemic tropical and subtropical regions elsewhere in the world.<sup>123</sup> Few cases reported outside the endemic areas were proven to be autochthonous<sup>124</sup> before the emergence of *C. gattii* infection in immunocompetent human and animal populations on Vancouver Island.<sup>45</sup> The incidence in this new endemic area has reached 36 cases/million population/year, markedly higher than rates reported in Australia (1.2 cases/million people/year).<sup>37,123</sup> Compared to *C. neoformans* infections, cryptococcosis caused by *C. gattii* is associated with a lower mortality rate, but is characterized by more severe neurologic sequelae due to the formation of granulomas that require surgery and prolonged therapy.<sup>123</sup>

Among *C. gattii* isolates, serotype B is more frequent worldwide than serotype C in causing infections, with the exception of sub-Saharan Africa.<sup>125</sup> Among *C. neoformans*, serotype A predominates, with the exception of some European areas where serotype D is prevalent and serotype AD also occurs.<sup>117,126</sup>

Several DNA-based methods, such as electrophoretic karyotyping, restriction fragment length polymorphism, random amplified polymorphic DNA, PCR fingerprinting, and multilocus enzyme electrophoresis, have been applied to distinguish the varieties and serotypes of *C. neoformans* or to discriminate among the strains. These typing methods have proven to be useful for epidemiologic investigations, providing evidence that, in most cases, a single strain or, more rarely, a second strain is involved in the recurrence or relapse of the disease.<sup>127–129</sup>

## Risk factors

Normal hosts are rarely reported to be infected with *C. neoformans* and, sometimes, a careful immunologic study of such patients can reveal subtle defects in their immunity that may have predisposed them to cryptococcosis. In contrast, normal or immunocompetent individuals may develop cryptococcosis due to *C. gattii* in those countries where this species is endemic. Cryptococcosis is uncommon in children, with a prevalence of up to 1% in those affected with AIDS.<sup>130</sup>

Diseases and therapies that impair host immune defenses predispose to cryptococcal infection. In the pre-AIDS era, major risk factors were lymphoproliferative disorders, corticosteroid therapy, sarcoidosis, organ transplantation and diabetes mellitus. Among neoplastic disorders, lymphoproliferative malignancies, mainly Hodgkin's lymphoma, are known to be the major predisposing diseases.<sup>131</sup> Delayed diagnosis together with immunosuppressive therapy and the clinical stage of malignancy contribute to the poor prognosis of cryptococcosis in these patients. Cryptococcosis remains extremely rare among patients with solid tumors.

Among organ transplant recipients, the incidence of cryptococcosis remains under 3% in most centers with no difference among the transplanted organs.<sup>132</sup> The type of primary immunosuppression after organ transplantation may influence the predominant clinical manifestation: patients receiving tacrolimus were less likely to have CNS involvement and more likely to have skin, soft tissue or osteoarticular involvement than patients who received other types of immunosuppression.<sup>132</sup> The time to onset varies significantly for different types of transplanted organ, with an early onset in lung (3 months) and late onset in heart and kidney (up to 12 years). An overall mortality rate of 20–50% is reported.

## Clinical manifestations

Primary cryptococcal disease almost always occurs in the lungs, following inhalation of the infectious propagules of the yeast. The disease can remain localized or disseminate via the bloodstream to other tissues, mainly to the CNS, even with resolution of the initial lung lesion.

The clinical picture of cryptococcal infection mainly depends on the host's immunodefenses. In patients with AIDS and in those treated with high doses of steroids and immunosuppressive drugs, the disease is associated with a higher fungal burden as a consequence of the low inflammatory response. With the introduction of HAART a new clinical manifestation, associated with exaggerated inflammatory reaction following immuno-reconstitution, has been described in AIDS patients treated with HAART soon after diagnosis of cryptococcosis.<sup>133</sup> The immune reconstitution inflammatory syndrome (IRIS) is defined as worsening of signs and symptoms occurring after an initial response despite ongoing appropriate antifungal therapy while the fungus is no longer cultured from any body site. A similar syndrome has also been described in solid organ transplant recipients with cryptococcosis.<sup>134</sup>

## Lung

In the immunocompetent host, inhalation of the fungus initiates a variety of clinical features.<sup>135</sup> Asymptomatic or mildly symptomatic pulmonary disease may develop, and resolve spontaneously or result in an encapsulated, usually non-calcified lung nodule. The lung can be the only site of involvement, and the nodule may be incidentally discovered on chest x-rays taken for other reasons. Its fungal etiology is recognized only if the nodule is aspirated or removed to exclude malignancy. Although rare, asymptomatic colonization of the respiratory tract may occur in patients affected with chronic obstructive pulmonary disease or cancer. Patients who develop



progressive pulmonary cryptococcosis usually present with chronic cough, low-grade fever, chest pain, scant mucoid or bloody sputum, malaise, and weight loss. The clinical course is subacute or chronic and may be complicated by concomitant extrapulmonary infections.<sup>136,137</sup> Abnormal chest radiographs of cryptococcal pneumonia in apparently immunocompetent hosts include single or multiple nodules, discrete bilateral infiltrates, hilar and mediastinal lymphadenopathy and, occasionally, pleural effusion and more rarely cavitation.<sup>138</sup>

In the immunocompromised host, patterns of diffuse lung changes are prevalent and dissemination frequently occurs. The clinical picture of disseminated cryptococcosis, with concomitant lung involvement, is almost always observed in AIDS patients who usually present at the hospital suffering from symptomatic meningitis. Primary pulmonary cryptococcosis is frequently unrecognized because of non-specific symptoms, and the importance of its early treatment is undervalued since only extrapulmonary cryptococcosis is considered for the definition of AIDS.

Some reports have proven that, in this patient population, primary pulmonary cryptococcosis is much more common than generally believed. In two series, a prevalence of primary pulmonary cryptococcosis as high as 39% and 78% was reported among patients affected with AIDS and cryptococcosis.<sup>108,139</sup> Fever, cough, dyspnea and pleural pain are the common initial manifestations. Typically radiographic features include diffuse or focal interstitial infiltrates with or without lymphadenopathy. Pulmonary nodules and alveolar infiltrates are uncommon.<sup>108,140,141</sup> Radiographic features of cryptococcal pneumonia are not specific and diffuse interstitial infiltrates are usually presumptively diagnosed as *Pneumocystis* pneumonia. Mild to moderate hypoxemia, but also acute respiratory failure (ARF), can occur. A prevalence of 14% of ARF associated with diffuse interstitial cryptococcal pneumonia was reported in one study focused on this complication.<sup>142</sup> The clinical course was found to be identical to that of *Pneumocystis* pneumonia and mortality was 100% with a median survival of 2 days.

The potential for diagnostic confusion and the co-existence of multiple opportunistic pathogens in patients with radiographic interstitial infiltrates reinforce the need for bronchoscopic confirmation of the diagnosis.<sup>108</sup> Examination of transbronchial biopsy and bronchoalveolar lavage (BAL) has proven effective in diagnosing 80–100% of cases.<sup>108,139,143,144</sup> Cryptococcal antigen detection in serum can also be helpful while awaiting culture results. Furthermore, attention should

be addressed to unexplained pleural empyema or effusion, and pleural fluid should be tested for the presence of cryptococcal antigen.<sup>145</sup>

The natural history of untreated primary pulmonary cryptococcosis in HIV-infected patients was shown by an African study to be “systemic dissemination within a few months of diagnosis.”<sup>139</sup> It is therefore mandatory to pay attention to primary pulmonary cryptococcosis in HIV-positive patients and promptly initiate specific treatment to halt the progression of this life-threatening disease.

## Central nervous system

*Cryptococcus neoformans* and *C. gattii* are strongly neurotropic and tend to disseminate from a primary, asymptomatic or manifest pulmonary focus to the CNS, primarily invading the leptomeninges. The infection may also extend to the brain's parenchyma to form massive lesions or mucoid cysts. The most common clinical form is meningitis, or meningoencephalitis, which seems to be a more appropriate term since the underlying brain parenchyma is often involved. Signs and symptoms in patients with and without AIDS are similar and include headache, fever, meningismus, visual disturbances, abnormal mental status and seizures.<sup>140,146-149</sup>

The clinical course, however, is somewhat different in AIDS versus non-AIDS patients. In patients with AIDS, the mycosis is characterized by a shorter duration of symptoms prior to presentation. Due to the poor immunoresponse of the host, symptoms usually appear late in the course of meningeal disease, when the fungal burden in the brain is high and the infection has spread to other organs and tissues. Coma may suddenly occur, sometimes associated with respiratory arrest. In contrast, in non-AIDS patients the onset is insidious and symptoms such as nausea, dizziness, irritability, decreased comprehension, impaired memory and unstable gait may begin many months or years before diagnosis is made. Symptoms may have a slow chronic course and nuchal rigidity and altered consciousness may appear gradually. Double vision and a blind spot in the visual field may be noted. Fever is often low grade or absent until late in the course of the infection. Headaches may be intermittent and their presence may be helpful in terms of diagnosis.<sup>150</sup> CSF features in AIDS and non-AIDS patients with cryptococcal meningitis are quite similar, but a reduced inflammatory response and higher fungal burden is often present in HIV-infected patients<sup>151</sup> (Table 9-2).

**Table 9-2** CSF findings in AIDS and non-AIDS patients with cryptococcal meningitis\*

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Computed tomography (CT) and the even more sensitive magnetic resonance imaging (MRI) are important tools for the diagnosis and management of cryptococcal meningoencephalitis. The radiographic appearance of CNS can vary according to the immunosuppression of the patients and the infecting fungus. In non-AIDS patients with meningitis, CT imaging can either be normal (50% of patients) or may reveal hydrocephalus (25%), gird enhancement (15%), single or multiple nodules (15%).<sup>152</sup> A normal scan may be found in half of the HIV-infected patients. Abnormal images include a diffuse cortical atrophy (34%), mass lesions (11%), hydrocephalus (9%) and diffuse cerebral edema (3%).<sup>153</sup>

Cerebral cryptococcoma is rarely caused by *C. neoformans* infections, but it remains the most common clinical form in the immunocompetent host infected with *C. gattii* strains.<sup>123</sup> Brain cryptococcoma, occurring in the absence of extracerebral disease or meningitis, are often suspected to be cerebral tumors on the basis of CT scans and/or MRI. Diagnosis always requires surgery. Image-guided nuclear magnetic resonance spectroscopy (NMRS) has been proposed as an unequivocal diagnostic technique to avoid neurosurgical biopsy. In fact, cerebral cryptococcoma can be identified by NMRS signals arising from the cytosolic trehalose released by the cryptococci present in the mass lesion.<sup>154</sup> An improved yield of the NMRS was obtained using a computerized analysis of the NMR spectra.<sup>155</sup>

In patients with increased intracranial hypertension at diagnosis or during treatment, CT or MRI scans may discriminate cerebral edema from hydrocephalus as in the former, ventricles are small or normal in size and sulci are flattened.<sup>156</sup>

Focal lesions and hydrocephalus are rare events in AIDS patients. The intracranial hypertension that affects most of these patients was shown to be related to cerebral edema; CSF hypertension, with a lumbar opening pressure of >250 mmH<sub>2</sub>O, is present in more than 50% of AIDS patients and is associated with a high fungal burden in the absence of inflammatory response. It was postulated that the massive amount of capsular polysaccharide might interfere with the normal egress of interstitial fluid into the subarchnoid space, causing cerebral edema.<sup>115</sup> Headache, papilledema, meningismus and hearing loss are the main symptoms.

## Eyes

Ocular infections or complications of cryptococcal meningitis are increasingly recognized and their manifestations include keratitis, papilledema, scotoma, chorioretinitis and ocular palsy, which often lead to irreversible visual loss.

The intraocular infection, namely chorioretinitis and vitritis, is the result of the infiltration of yeast cells from the subarachnoid space or of hematogenous spread from other infected sites.<sup>157</sup> Ocular involvement in some cases precedes symptomatic meningitis. Cryptococcal chorioretinitis is similar to *Candida* infection but usually presents with few lesions. Diagnosis is made by examination of aqueous or vitreous humor.

Two processes leading to loss of vision have been described that may develop in the absence of ocular lesions in patients with cryptococcal meningitis:<sup>158,159</sup>

- a “rapid” visual loss, due to optic neuritis caused by invasion of the optic nerve by the pathogen, occurs in 12–24 hours, or early in the course of therapy

- a “slow” progressive visual loss, related to the increase of intracranial pressure, may occur during treatment for cryptococcal meningitis and shunt or optic nerve fenestration may halt the progression of visual loss.

Factors that predict visual loss are papilledema, elevated CSF opening pressure and a positive CSF India ink preparation.<sup>159</sup> Recovery of vision is uncommon, so early recognition and treatment are essential to prevent permanent sequelae.

Keratitis has rarely been reported subsequent to corneal transplantation.<sup>101,160</sup>

## Lymph nodes

Occasionally, lymph nodes may be the only apparent site of the infection, particularly in HIV-infected patients. The cervical or supraclavicular lymph nodes are the most involved.

Fine needle aspiration of enlarged lymph nodes in high-risk groups has been proven to be an excellent diagnostic procedure in terms of yield and rapidity of isolation of the fungus and for detecting its presence in cytologic preparations.<sup>161</sup>

Lymphadenitis is reported as the most common presentation of cryptococcal IRIS, postulated to be due to an inflammatory response to cryptococcal antigens present in the lymph nodes.<sup>162</sup>

## Skin

Although primary cutaneous lesions following a direct inoculation of the fungus into the skin are reported,<sup>109,110</sup> in the great majority of cases skin lesions result from the hematogenous dissemination of the infection.<sup>112</sup> Skin lesions are estimated to occur in at least 10–15% of patients, mostly affected with AIDS, sarcoidosis and those receiving high-dose corticosteroids.<sup>117</sup>

A painless papule with central softening is usually the initial lesion. Then, multiple and extremely varied lesions can follow such as nodules, ulcers, purpura, acneiform lesions, abscesses, granulomas, plaques and lastly, associated with AIDS, cellulitis, herpetiform and molluscum contagiosum-like lesions.<sup>112</sup> The impressive variety of cutaneous manifestations emphasizes the need to perform histology and culture of biopsied tissues from any new skin lesion presenting in high-risk patients, since diagnosis of the infection or of concomitant infections may be facilitated.

## Bone

Cryptococcosis of the bone is an uncommon but severe infection that is rarely diagnosed at presentation as it may be confused with other infections or with a neoplastic disease. Osteomyelitis affects immunocompetent hosts as well as immunocompromised patients, particularly those with sarcoidosis, and it is remarkably rare in patients with AIDS, although bone marrow involvement has been observed.<sup>163</sup> The vertebrae and bony prominences are the most involved sites. The infection may be acquired by hematogenous spread from a self-limited pulmonary or lymph node localization, or may originate from a contiguous skin lesion. In addition, cases of temporal bone cryptococcosis have been shown to be subsequent to meningitis.<sup>164</sup> Cryptococcal arthritis also may occur, usually as an extension of the infection from the contiguous bone into the joint space.

Symptoms may be absent, despite abnormal radiographs, or may be present as soft tissue pain and swelling or as general symptoms of the disseminated infection. Radiographs uniformly reveal osteolytic and eroded lesions consistent with areas of bone destruction associated with abscesses containing mucoid, gelatinous pus. At histology granulation tissue with giant cell granulomata is evidenced. Diagnosis can be made by aspiration, or by incision and drainage, or by surgery. Serum antigen tests are positive in approximately half of the patients.

## Other foci of infection

In disseminated cryptococcosis, any organ or tissue may have foci of infection. The prostate has been shown to be an asymptomatic reservoir of the infection, particularly in AIDS patients. Seminal fluid and urine, collected after prostate massage, were found to contain living cryptococci, and the infection may persist after successful treatment of the disseminated disease.<sup>165</sup> Thus, in cases of discontinuation of maintenance treatment, the prostate should be checked for persistence of the infection.

## Laboratory diagnosis

### Serology

Detection of polysaccharide antigen in body fluids is highly effective for a rapid and accurate diagnosis. The most frequently used test is the slide agglutination using latex particles (LA), coated with polyclonal antibodies<sup>166</sup> or with anti-glucuronoxylomannan monoclonal antibodies.<sup>167</sup> Both polyclonal and monoclonal antibodies can detect up to 10 ng of polysaccharide per ml of biologic fluid. A positive serum antigen test at a dilution of 1:4 is strongly suggestive for cryptococcal infection, and a titer of  $\geq 1:8$  is indicative of active disease. In general, higher antigen titers indicate more severe infections and a falling titer is a good prognostic sign. False-negative test results are unusual and can be due to a prozone effect or immune complexes, or low production of antigen.<sup>168,169</sup>

Several commercial kits are available. Kits for LA exhibit a specificity ranging from 93% to 100%. Sensitivity for CSF samples ranges from 93% to 100% and for serum samples from 83% to 97%.<sup>170,171</sup> The sensitivity of the different kits has been shown to vary depending on the pretreatment of serum samples with pronase that reduces prozone reaction, enhancing antigen detection and increasing titers.<sup>168,172,173</sup> CSF specimens, however, do not require pronase treatment.<sup>168,173</sup> False-positive reactions with serum or CSF may be caused by rheumatoid factor and can be recognized by testing the sample with latex particles sensitized with normal rabbit globulins. Pretreatment with pronase, with reducing agents (2- $\beta$  mercaptoethanol or dithiothreitol) or boiling for 5 minutes with EDTA also eliminates false positives due to rheumatoid factor and other unknown factors. Care should be taken to avoid contamination of specimens with surface condensation from agar media or talc from latex gloves, and to thoroughly remove disinfectants and soaps used for cleaning slides that may cause false-positive reactions.<sup>174,175</sup> Cross-reactions have been reported with *Trichosporon beigeli*<sup>176</sup> and with the bacterium DF-2 (*Capnocytophaga canimorsus*).<sup>177</sup>

**Table 9-3** Percentage of CSF positivity in patients with cryptococcal meningitis

	Non-AIDS patients	AIDS patients
Positive antigen	86–95%	100%
Positive India ink	50%	82%
Positive culture	90%	100%

Cross-reactions with *Cryptococcus albidus* have also been reported and may be helpful in diagnosis of infection due to this species.<sup>178</sup> Commercial kits cannot be used interchangeably to monitor changes of antigen titers in patients. Each laboratory should select and employ a kit from only one manufacturer and check each new batch with reference reagents.

An enzyme immunoassay (EIA) kit has been developed that utilizes a polyclonal capture system and a monoclonal detection system.<sup>179</sup> This test has some advantages over the LA test. It is more sensitive and provides earlier detection, is unaffected by prozone reactions, does not require treatment with pronase and does not react with rheumatoid factor. In addition, the reading is less subjective than that of LA. The LA and EIA titers are not equivalent. Thus, EIA titers cannot be converted into LA titers and vice versa.<sup>179</sup>

The antigen detection test is highly specific and more sensitive in comparison to microscopy and culture (Table 9-3), and it may be the only positive test when used for screening or early diagnosis. Antigen titers are much higher in AIDS patients than in HIV-negative individuals. In AIDS patients, CSF antigen titer  $\leq 1:2048$  at baseline is predictive of a more favorable outcome.<sup>149,180</sup> Patients with elevated and stable CSF and/or serum antigen titers, despite culture conversion to negative, are likely to relapse. The test is also useful for monitoring the clearance of antigen during maintenance therapy.<sup>181</sup>

The value of positive antigenemia in the absence of isolation of the fungus has been questioned and considered unreliable. But patients with apparent isolated antigenemia, when monitored after the first positive antigen test, have been shown to develop the infection that was detectable by microscopy and culture.<sup>182</sup> Therefore, positive serum or CSF cryptococcal antigen titers should be taken into serious consideration<sup>182,183</sup> and procedures to enhance the isolation of the fungus should be adopted.<sup>144,184,185</sup> Isolation of the yeast is essential to confirm diagnosis and determine its in vitro antifungal susceptibility.

Tests for the detection of anticryptococcal antibodies are not useful for diagnosis since, during active infection, capsular polysaccharide may inhibit antibody synthesis or may mask antibody presence. On the contrary, antibodies have been proven to have a prognostic value in non-AIDS patients during recovery from active infection when antigen titers decline.<sup>186,187</sup>

### Microscopy

In direct examinations of wet mounts the capsule of the yeast is usually not visible with light microscopy unless the organism is observed in a suspension of India ink. The ink's carbon



particles do not penetrate the capsule which appears as a clear halo surrounding the yeast cells. India ink examination of CSF is the most rapid test for diagnosing cryptococcal meningitis. In addition, it may give evidence of a high fungal burden, which should alert clinicians to the risk of an increase of intracranial pressure following the start of antifungal therapy.

The sensitivity of the test depends on the volume of sample examined and may be improved by examining the pellet from the centrifuged CSF. Cryptococci may be distinguished from lymphocytes and artefacts by the presence of a clear refractive cell wall and characteristic buds. In addition, the halo that may be present around the lymphocytes progressively reduces and disappears in 5–10 minutes. India ink is easily contaminated, so periodic renewal of the ink is required.

*Cryptococcus neoformans* may not be recognized in dried smear preparations of biologic samples because the fungal cells collapse, become crescent shaped and stain irregularly. Yeast cells can also be stained with Loeffler's methylene blue, toluidine blue or Wright's stain. Gram staining is variable, ranging from intensely purple to shades of pink.

In tissue sections stained with hematoxylin and eosin, the yeast is eosinophilic or lightly basophilic. The yeast cells are uninucleate, thin-walled, spherical, oval to elliptical in shape, and 2–20  $\mu\text{m}$  in diameter.<sup>188</sup> Single buds with a narrow base are common. Identification of cryptococci in tissues requires specific stains for fungi such as the periodic acid-Schiff (PAS) and Grocott methenamine silver stains which, however, do not reveal the presence of capsules. With Mayer's mucicarmine the polysaccharide capsule is well stained and rose to burgundy in color. Another mucin stain, the Alcian blue colloidal iron stain, can be used to detect the capsule. Capsule-deficient cryptococcal cells in tissue sections are difficult to differentiate from other yeasts, unless the Fontana-Masson stain, which colors the fungal cell wall's melanin dark brown or black, is used.<sup>189</sup>

The corpora amylacea in aged brains and Michaelis-Gutmann bodies, associated with malakoplakia, resemble capsule-deficient cryptococci, due to their PAS-positive staining.

The host reaction against cryptococcal cells depends on the patient's underlying disease and immunocompetence, and the yeast cell's capsule. In anergic patients, the inflammatory reaction is poor and the yeast actively multiplies to form cystic lesions filled with highly encapsulated cells that displace the surrounding tissues. In immunocompetent subjects, focal suppurative and necrotizing inflammatory reactions develop, which may lead to resolution of the infection or evolve into a granulomatous and eventually nodular and fibrocaceous lesion. Infection with a capsule-deficient strain is usually diagnosed in immunocompetent individuals and is often localized to the initial site of entry, such as the lungs or skin. The granulomatous reaction consists of epithelioid histiocytes and multinucleated giant cells with ingested acapsulated yeasts.

## Culture

Cryptococci can be cultured from biologic samples on most standard media used for laboratory diagnosis, after 48–72 h incubation at 30–35°C in aerobic conditions. Antibacterial antibiotics should be added to the media. The yeast does not grow in the presence of cycloheximide at the concentrations used in selective isolation media, and incubation temperatures  $\geq 37^\circ\text{C}$  should be avoided in order to assure fungal isolation.

Cultures should be held for 3–4 weeks prior to discarding, in particular if the patient is under antifungal treatment.

As only a few yeast cells may be present at the site of the infection, pellets from centrifuged CSF, blood and other biologic fluids should be cultured. Bronchial secretions and urine, especially from AIDS patients, are often contaminated by *Candida* species which, by its rapid growth, masks or inhibits the growth of cryptococcal cells.

The differential medium, niger seed agar, as a primary culture medium can be used along with Sabouraud dextrose agar to recognize, among the white *Candida* species colonies, *Cryptococcus* colonies that turn brown by their ability to break down caffeic acid to melanin.<sup>52</sup> The same medium can be used to culture membrane filter pads (pore size of 1.2  $\mu\text{m}$ ) through which a large amount of urine has been filtered.<sup>185</sup>

A selective medium, inositol agar with chloramphenicol, has also been developed to inhibit *Candida* growth and enhance isolation of *Cryptococcus* species.<sup>190</sup> On this medium pellets from centrifuged bronchial secretions and urine can be inoculated.<sup>144</sup> Inositol, as the unique carbon source, is assimilated by *Cryptococcus* species but not by *Candida* species that may be present in biologic fluids. After 3–5 days of incubation, *Cryptococcus* colonies can be recognized among the pinpoint *Candida* colonies, which develop as residual growth.

## Identification

*Cryptococcus* yeast cells usually grow on conventional isolation media with small or no capsule. However, they can be easily identified as *Cryptococcus* species as they do not produce hyphae or pseudohyphae, are not fermentative, assimilate inositol and hydrolize urea. A rapid test for urease activity has been developed that becomes positive within 15 minutes.<sup>191</sup> In contrast, other urease-positive species of yeasts from clinical specimens require more than 3 hours. *C. neoformans* isolates lacking urease or phenoloxidase activity have been rarely reported.<sup>192,193</sup>

Occasionally some *Cryptococcus* species, other than *C. neoformans* and *C. gattii*, may be isolated from clinical specimens as colonizers or contaminants. Except for a few clinical cases due to *C. albidus*, *C. curvatus* and *C. laurentii*, other *Cryptococcus* species have not been shown to cause disease. Table 9-4 shows the differential biochemical characteristics of the *Cryptococcus* species that are able to grow at 35°C and can cause disease. Two agar media, CGB agar and D-proline agar, can be used to distinguish *C. gattii* from *C. neoformans*.<sup>29,33</sup> Up to now serotyping has been carried out with the slide agglutination test using antibodies specific for the capsular polysaccharide.<sup>23</sup>

In the absence of serotyping reagents, discrimination among serotypes is also possible by molecular methods that also allow the mating type identification.<sup>194-198</sup>

## In vitro susceptibility testing

In vitro susceptibility testing has proven useful to aid clinical decisions about treatment regimens and dosage selections. *C. neoformans* strains isolated before treatment are usually susceptible to amphotericin B and, in most cases, to flucytosine and azoles. But development of resistance during treatment, although exceptional in respect to amphotericin

**Table 9-4** Differential characteristics of *Cryptococcus* species able to grow at 35°C

Species	Growth at 37°C	Assimilation			
		Nitrate	Lactose	Melibiose	Glycerol
<i>C. neoformans</i>	+	-	-	-	-
<i>C. gattii</i>	+	-	-	-	-
<i>C. albidus</i>	-	+	v	v	v
<i>C. curvatus</i>	-	-	+	-	+
<i>C. heveanensis</i>	-	-	+	-	-
<i>C. humiculus</i>	v	-	+	+	+
<i>C. laurentii</i>	v	-	+	+	v

v, variable.

B,<sup>199</sup> may occur with flucytosine when the drug is given in monotherapy,<sup>200</sup> and to fluconazole, because of its extensive use in AIDS patients for prophylaxis or chronic therapy.<sup>201-203</sup> Cross-resistance to itraconazole usually does not occur in *C. neoformans*, possibly due to the dual target activity identified for this azole, namely the lanosterol 14  $\alpha$ -demethylase enzyme and the NADPH-dependent 3-ketosteroid reductase.<sup>204</sup>

A global survey based on in vitro testing of more than 1800 clinical isolates confirms that in vitro resistance remains an uncommon event.<sup>205</sup>

Although no breakpoint has yet been defined for any drug,<sup>206-213</sup> it has been assumed that an infection caused by a strain with an amphotericin B MIC greater than 1  $\mu\text{g/ml}$  will not respond to conventional amphotericin B treatment. Strains with a flucytosine MIC of  $\geq 32 \mu\text{g/ml}$  are considered resistant to this drug, and strains with a fluconazole MIC of  $\geq 16 \mu\text{g/ml}$  also are not likely to respond to this antifungal in monotherapy with standard dosing schedules. A positive interaction has been proven in vitro for flucytosine with amphotericin B or azole, or with both.<sup>208,213,214</sup>

## Therapy

According to its natural history, cryptococcal meningitis is always fatal if untreated. The introduction of amphotericin B deoxycholate (D-AmB) in the 1950s improved prognosis of this disease and cure rates for cryptococcal meningitis rose to over 50%, but substantial toxicity was reported. Subsequently, flucytosine was developed and, because of its excellent CSF penetration and great activity against the yeast, it was initially used as a single agent to treat meningitis. Its use in monotherapy, however, was associated with the development of resistance and consistent high numbers of failure.<sup>200</sup>

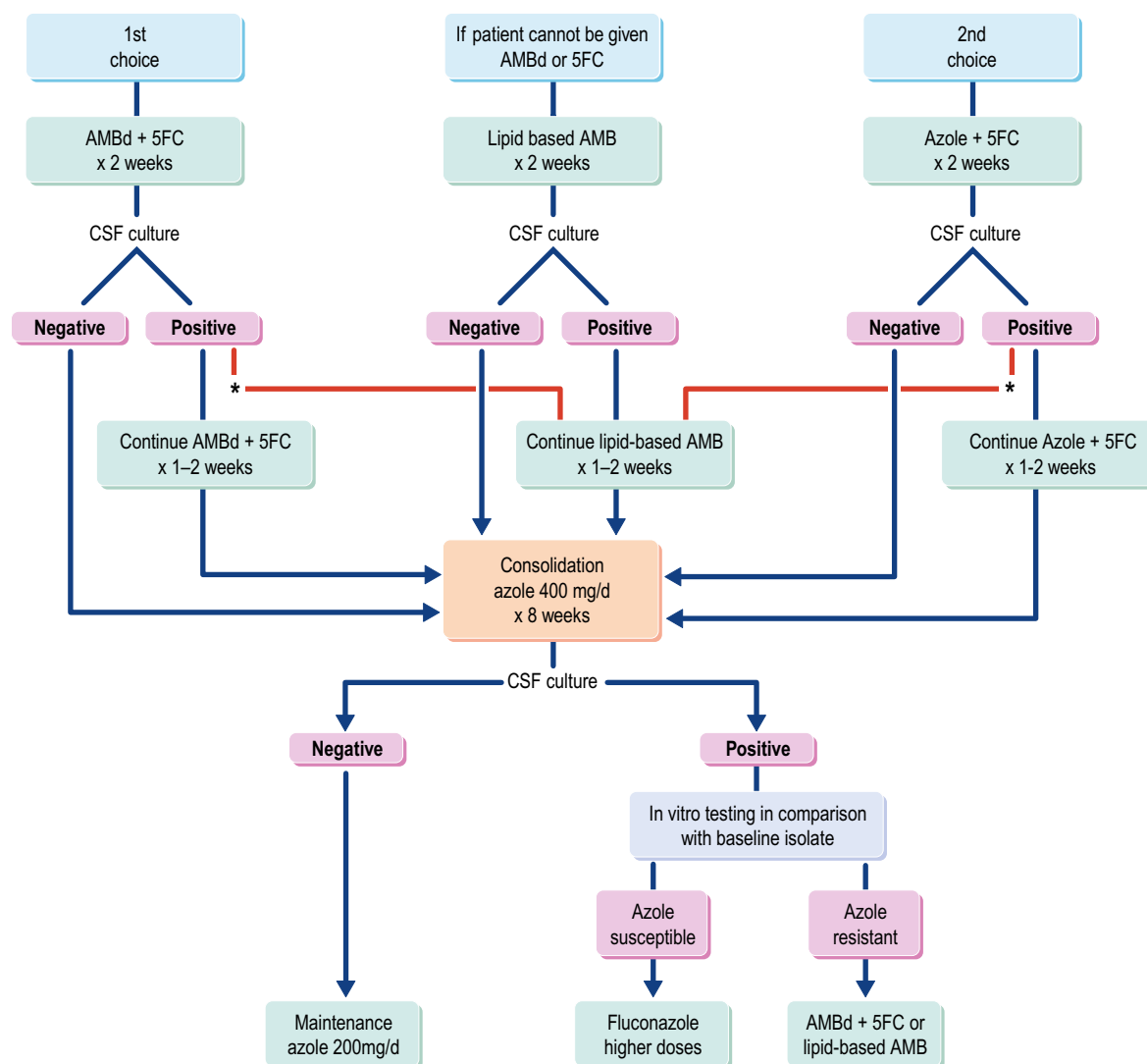
Progress in the management of cryptococcal meningitis was achieved when flucytosine was combined with D-AmB, to take advantage of the synergistic or additive mechanism of action and complementary pharmacokinetics of the two antifungals. The combination therapy allowed a reduction in the amount of D-AmB needed to treat cryptococcosis, thus limiting its

toxicity; also it reduced concerns about the development of resistant strains to flucytosine.<sup>215</sup>

In two pivotal studies carried out in the pre-AIDS era, issues were addressed to achieve, with the combined therapy, the highest success rate with the lowest toxicity. Six-week combination therapy was first compared with 10-week D-AmB monotherapy and in the second trial the combination regimen was compared with the 4-week combination.<sup>150,215</sup> In the second trial, despite the increase in the success rates to approximately 80%, the rates of side effects were still too high with both regimens and, in particular, a subset of severely immunosuppressed patients failed the short course regimen. The insights from this study highlighted two problems: the need to further shorten the initial combined therapy, which was shown to be able to sterilize the CSF in 2 weeks, and the need to continue treatment with a single agent to consolidate the clinical and mycologic responses achieved with the induction therapy.

Significant pretreatment predictors of favorable response in patients with cryptococcosis have been identified as the ability to control the underlying disease, normal mental status with or without headache and, in AIDS patients a low CSF fungal burden evidenced by an antigen titer  $\leq 1:2048$ .<sup>149,150,180</sup>

After the emergence of the AIDS epidemic, carefully conducted prospective randomized clinical trials and retrospective surveys evaluated D-AmB and the new azoles in monotherapy and in combination with flucytosine for the treatment of meningeal cryptococcosis.<sup>147,216-221</sup> The clinical use of D-AmB in combination with flucytosine as primary therapy is now recommended as the first choice in HIV-negative patients with cryptococcal meningitis and in moderate to severe case in patients with AIDS (Fig. 9-1). A strategy of 2-week induction therapy followed by 8–10 week consolidation with fluconazole proved to be successful in AIDS patients. If fluconazole is not an option, an acceptable alternative is itraconazole<sup>220,222</sup> (Table 9-5). It seems reasonable to follow the same strategy for HIV-negative patients with cryptococcal meningitis. Both azoles have made a significant impact on the management of cryptococcosis and they have been effectively used to treat cryptococcal meningitis in animals and humans despite their different CSF pharmacokinetics.<sup>149,223,224</sup>



**Figure 9-1** Treatment of cryptococcal meningitis in patients with AIDS.

In a large randomized clinical trial, the use of 400 mg/day of itraconazole or fluconazole for consolidation therapy in AIDS patients with cryptococcal meningitis showed a similar efficacy. Symptoms were suppressed in 70% and 68% of patients and CSF sterilized in 92% and 97%, respectively.<sup>220</sup>

Since eradication of the infection could not be achieved in AIDS patients in the pre-HAART era, life-long maintenance therapy was required to prevent relapse.<sup>147,225</sup> Comparative data between fluconazole and itraconazole showed fluconazole superior to itraconazole, with a relapse rate of 4% vs 23%.<sup>226</sup> The lower efficacy of itraconazole was in part attributed to the absence of flucytosine during the initial 2 weeks of primary treatment; this was the factor best associated with relapse (relative risk, 5.88). In addition, itraconazole activity may have been impaired by unpredictable oral absorption of capsule formulation and the more frequent interactions with other drugs. The possibility of discontinuing the chronic suppressive antifungal therapy has been indicated in selected patients who remain free of cryptococcal disease and have a sustained increase in their

CD4 lymphocyte counts to >100–200 cells/ml after HAART.<sup>227</sup> Antifungal therapy should start again if there is a decrease of CD4 count below 100 cells/ml or in case of evidence of relapse.

In non-AIDS patients, life-long maintenance treatment following completion of primary therapy has never been suggested, since eradication of the infection may be achieved. However, relapse was reported in up to 27% of patients, mostly within 3–6 months after discontinuation of therapy.<sup>150</sup> Thus, it should be reasonable to consider a prolonged consolidation treatment with an azole for 6 months and possibly up to 1 year associated with a close follow-up.

Mycologic surveillance of treatment efficacy requires that lumbar punctures be performed:

- at the end of the first 2 weeks of induction therapy, to ensure sterilization of the CSF
- at the end of the consolidation therapy
- whenever indicated by a negative change in the clinical status of the patient, during the follow-up period.



**Table 9-5** Treatment of cryptococcal meningitis in patients with AIDS

<b>Primary therapy*</b>
i) 2-week initial therapy flucytosine 100 mg/kg/d in 4 doses plus AmB 0.7–1 mg/kg/d
ii) 8–10 week consolidation therapy fluconazole (loading dose 800mg/d for 2 days followed by 400mg/d)
or itraconazole (loading dose 600mg/d for 3 days followed by 400mg/d in 2 doses)
<b>Maintenance therapy</b>
fluconazole 200mg/d **
or itraconazole 200 mg/d ***
*Data from Saag et al. <sup>222</sup>
**Data from Saag et al. <sup>226</sup>
***Data from Viviani, <sup>224</sup> de Gans et al, <sup>243</sup> de Lalla et al. <sup>244</sup>

Failure to achieve negative CSF culture by day 14 is an indication of a much higher chance of failure of the consolidation therapy.<sup>180,220</sup> The LA test has been considered of limited prognostic value in AIDS patients. However, in other experiences the antigen titer parallels the clinical course of the disease even though an increase of the titer may be observed in the first 7–10 days of effective therapy and the subsequent decline may be more or less rapid on an individual basis.<sup>184</sup> High stable antigen titers suggest poor prognosis or high risk of relapse.

In patients who fail primary therapy or in those who cannot receive or tolerate D-AmB or flucytosine, a lipid formulation of AMB may be used as salvage therapy. Few comparative studies have been carried out with these formulations in humans. A study comparing D-AmB and amphotericin B lipid complex was not conclusive.<sup>228</sup> In contrast, in two comparative randomized trials, liposomal amphotericin B (AmBisome) at the daily dose of 3–4 mg/kg showed similar or higher activity, but significant less toxicity, than conventional D-AmB.<sup>229,230</sup>

Few non-comparative trials have been reported on the use of flucytosine associated with fluconazole, itraconazole or in triple therapy with D-AmB and fluconazole with a success rate of 75%, 92% and 90%, respectively.<sup>217,224,231</sup> No comparative data are available concerning treatment with the new azoles, voriconazole and posaconazole, that have shown to be very active in vitro against *C. neoformans*.<sup>205</sup>

Elevated intracranial pressure (>250 mmH<sub>2</sub>O) may require CSF drainage, which contributes to the successful outcome of treatment. For patients with elevated intracranial pressure in the lateral recumbent position, a lumbar puncture should be performed daily to remove a volume of CSF (up to 30 ml) sufficient to reduce the pressure to <200 mmH<sub>2</sub>O or 50% of the initial opening pressure until the opening pressure is consistently less than 250 mmH<sub>2</sub>O.<sup>115</sup> In cases of hydrocephalus, early shunt placement is suggested to avoid irreversible neurologic damage. CNS shunt or optic nerve fenestration may halt

progression of visual loss caused by the progressive increase of intracranial pressure.<sup>159</sup> Although the timing for placement is controversial, it seems reasonable to consider the placement of a shunt after the initiation of an effective antifungal therapy.<sup>156</sup> The use of steroids, acetazolamide or mannitol, although effective in decreasing cerebral edema, should be carefully evaluated because of the risk of worsening cryptococcal infection.

Brain cryptococcoma usually respond to the treatment regimen used for cryptococcal meningitis. Consolidation with monotherapy, however, may require continued treatment for up to 1 or 2 years. Enlargement of the cryptococcoma or appearance of new small lesions during initial therapy does not necessarily represent failure but may be the result of the inflammatory response against the fungus. Lesions will progressively decrease during treatment. Surgical resection is rarely needed and depends on individual cases.<sup>232</sup>

Fluconazole (≥400 mg/day) monotherapy for 6–12 months is recommended for mild cryptococcal disease.<sup>233</sup> However, attempts to identify those patients who would best respond to fluconazole-based regimens revealed that concomitant use of flucytosine strongly correlated with a better outcome.<sup>210</sup>

A consensus has not been reached on the optimal management of extraneural cryptococcosis. Debate is still ongoing on the need to treat an asymptomatic patient whose bronchial secretions contain cryptococcal cells. It seems advisable in the immunocompetent patient that pulmonary involvement is ruled out by a careful roentgenographic control and a mycologic examination of biologic samples before deciding not to treat. In patients with proven pulmonary cryptococcosis a lumbar puncture should be performed to exclude meningeal involvement.<sup>234</sup> When an immunocompromised patient shows *C. neoformans* in bronchial secretions, treatment is mandatory. In the case of isolation of the fungus in all other clinical settings, particularly in the immunocompromised host, guidelines for treatment of meningitis should be followed, considering that cryptococcosis is a fatal disease and may have an unpredictable course. Surgical resection is occasionally required for large pulmonary masses unresponsive to antifungal treatment. Although antifungal therapy alone can be successful in clearing pleural effusions, surgical drainage combined with antifungals may be beneficial in cases of gross empyema. In addition, surgical debridement combined with systemic antifungal treatment has been proven to be the optimal regimen in severe bone cryptococcosis. Radiologic monitoring showed resolution of bone lesions after 3 weeks to 30 months of therapy.<sup>163</sup>

To avoid the occurrence of cryptococcal IRIS, HAART should be delayed until such time as the antifungal treatment has markedly reduced the fungal burden.<sup>235</sup>

## Prevention

Patients at high risk of developing cryptococcosis can now be identified and tentative guidelines have been suggested, as follows.

- Minimize exposure to the fungus by avoiding (i) sites highly contaminated with avian excreta, in particular pigeon excreta; (ii) pet birds in living spaces, at home or at work places; (iii) activities related to pet care, such as cleaning cages or

breeding birds; (iv) staying in sites where numerous *Eucalyptus* species or other trees, proven to be the natural habitat of *C. gattii*, are present; (v) smoking that has been shown, in HIV-infected patients, to reduce the antifungal activity of alveolar macrophages<sup>236</sup> and is assumed to enhance deposition of the inhaled organism in the airways; (vi) contaminated air conditioning systems. Highly contaminated sites can be cleaned with an alkaline solution.<sup>237</sup>

- Prevent infection by administration of fluconazole to patients at high risk. Efficacy of this measure in reducing the incidence of cryptococcal infection has been proven, but drawbacks are represented by elevated cost, possible selection of resistant strains, and drug interactions. In addition, primary fluconazole prophylaxis is not supported by the incidence of the infection.<sup>238</sup>
- Early diagnosis by screening asymptomatic patients with low CD4+ count (<100/mm<sup>3</sup>) for cryptococcal antigen in serum.<sup>184,239</sup> Despite the remarkable sensitivity of the test and the low cost, compared to that of the primary prophylaxis or management of the disease, screening is not indicated for HIV-positive patients with a low prevalence of cryptococcosis.
- Several antigens have been identified that appear to be suitable vaccine candidates. A vaccine against cryptococcosis in humans is probably feasible but there are significant obstacles to vaccine development ranging from uncertainties about the pathogenesis of the infection to economic considerations.<sup>240</sup>

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# Infections caused by non-*Candida*, non-*Cryptococcus* yeasts

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Most yeast infections of humans are caused by species of *Candida* and *Cryptococcus*. However, there are other genera that have emerged as pathogens paralleling the increases in immunocompromised populations. The genera most likely to cause infections include *Malassezia*, *Trichosporon*, *Pichia*, *Rhodotorula*, *Saccharomyces*, *Sporobolomyces*, and *Blastoschizomyces*. *Trichosporon* and *Malassezia* species are frequently involved in superficial infections, but members of all six genera cause fungemia with or without organ invasion in compromised patients, often are resistant to antifungals and are a challenge to treat.<sup>1-6</sup>

These genera are a very heterogeneous group of fungi, some of which have undergone revisions in classification and nomenclature.<sup>7,8</sup> *Trichosporon*, *Malassezia*, *Rhodotorula*, and *Sporobolomyces* are basidiomycetous yeasts characterized by their urease activity, diazonium blue B (DBB) staining reactions, guanine/cytosine (G/C) content, DNA association rates, and RNA sequencing.<sup>7</sup> Species of these genera are often anamorphs (asexual states), *Sporobolomyces* spp. being the anamorph of the sexual genus *Sporidiobolus* and *Rhodotorula* being the anamorph of the sexual genus *Rhodospodidium*.

*Saccharomyces*, *Pichia*, and *Blastoschizomyces* are ascomycetous yeasts characterized by a lack of urease production and the formation of ascoconidia. *Saccharomyces* has a sexual cycle, some species of the teleomorphic genus *Pichia* have *Candida* anamorphs, and *Blastoschizomyces* has no known teleomorph. Although they are not recovered from clinical specimens as frequently as many *Candida* spp., they can be important pathogens. *Saccharomyces* is the most frequently encountered in clinical specimens, often as a colonizer,<sup>9</sup> whereas *Pichia* and *Blastoschizomyces* are uncommon but important causes of opportunistic infections.

## Malassezia

### Definition

*Malassezia* is characterized by yeast species that are lipophilic, and are isolated from the skin of warm-blooded animals and humans as commensals, but can also cause dermatologic diseases such as pityriasis versicolor. There are currently

11 species: *M. dermatis*, *M. furfur*, *M. globosa*, *M. japonica*, *M. nana*, *M. obtusa*, *M. pachydermatis*, *M. restricta*, *M. sloofiae*, *M. sympodialis*, and *M. yamatoensis*.<sup>8,10-15</sup> The main causative agents of pityriasis versicolor are *M. globosa* and *M. sympodialis* and occasionally *M. furfur* (formerly *Pityrosporum orbiculare*) and *M. sloofiae*.<sup>16-19</sup> *M. pachydermatis* is associated with animals and animal skin and has caused nosocomial outbreaks in neonatal intensive care units (NICU).<sup>20,21</sup>

### Epidemiology, clinical characteristics and treatment

Prior to 1990, only *M. furfur*, *M. pachydermatis*, and *M. sympodialis* were recognized.<sup>22-24</sup> Molecular taxonomic studies have divided *M. furfur* into six species: *M. furfur*, *M. sympodialis*, *M. globosa*, *M. obtusa*, *M. restricta*, and *M. sloofiae*.<sup>10,25</sup> Recently, four additional lipid-dependent species have been described.<sup>12-15,26,27</sup> *M. dermatis* and *M. japonica* from human patients with atopic dermatitis,<sup>14,15</sup> *M. yamatoensis* from human patients with seborrheic dermatitis,<sup>13</sup> and *M. nana* from animals (cats and cows)<sup>11,28</sup> have been studied. Throughout much of the literature *Malassezia* infections were reported as being caused by *M. furfur* or “*Pityrosporum ovale*” or “*P. orbiculare*” and cannot be correlated with currently accepted species.<sup>22</sup>

*Malassezia* species are members of the normal human cutaneous commensal flora and can be isolated from sebaceous-rich areas of the skin, particularly the chest, back, and head regions.<sup>29,30</sup> Skin colonization with the different species has been associated with patient age and geographic location.<sup>22,23</sup> *M. sympodialis* was common on the skin of healthy individuals in Spain and Japan.<sup>17,31,32</sup> *M. globosa* was the most common species on the scalp of adults and children in the UK, whereas *M. sympodialis* and *M. restricta* were also common in adults.<sup>23</sup> *M. furfur* was an uncommon colonizer in the UK, Spain and Japan but more common in tropical areas.<sup>22,23</sup> Colonization occurs less frequently among children compared to adolescents. Carriage of *Malassezia* appears to increase around puberty, correlating with increased sebaceous gland activity.<sup>33</sup> Colonization rates of healthy newborns are unknown, but rates in hospitalized neonates range from 37%<sup>34</sup> to 100%.<sup>35</sup> Young gestational age<sup>34,36</sup> and extended hospitalizations<sup>34,36-38</sup> may

predispose newborns to colonization. High colonization rates are an important risk leading to an increase of *Malassezia* catheter-related fungemia in premature neonates.<sup>22</sup>

As a colonizer, *Malassezia* is found in a budding yeast form. During infection, it undergoes transition from the yeast to the mycelial form, which can invade but still be confined to the dead stratum corneum.<sup>22,39-41</sup> The infection (pityriasis versicolor) is non-inflammatory, causing scaling of small areas of skin, often on the torso, shoulders, and upper arms (Fig. 10-1). Lesions may be hypo- or hyperpigmented and, apart from the skin discoloration, are asymptomatic. Rarely, deeper skin invasion occurs, leading to erythema and pruritus. Predisposing factors include a “genetic susceptibility,”<sup>42,43</sup> illness or malnutrition,<sup>42</sup> increased plasma cortisol level,<sup>42,44,45</sup> and high ambient temperature and

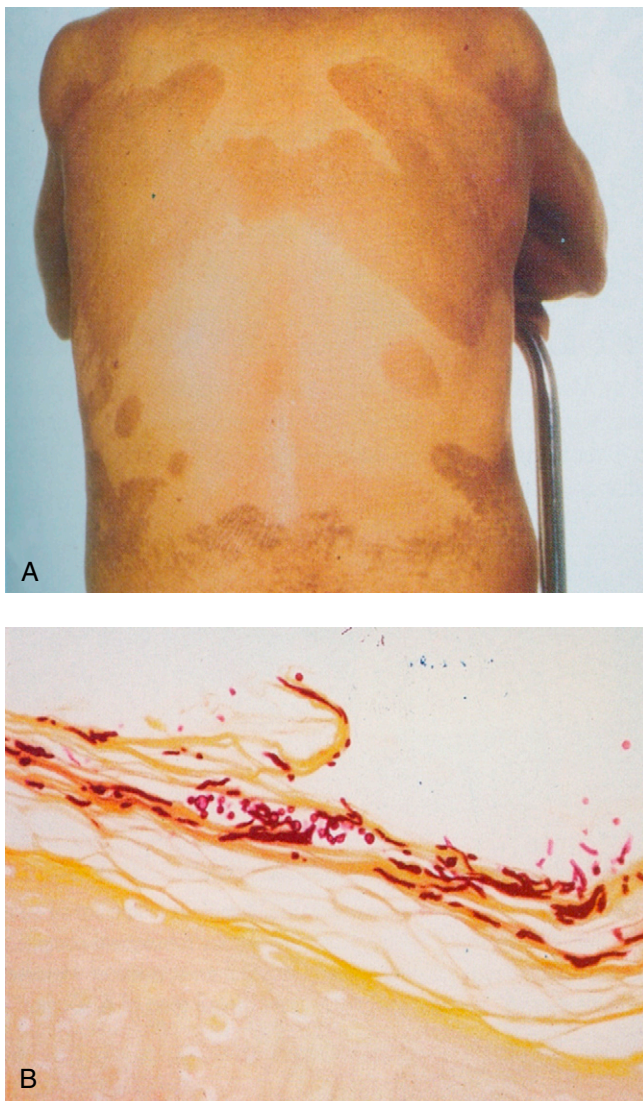
humidity.<sup>46</sup> It occurs worldwide but is encountered more frequently in tropical climates where incidences of 40–60% are reported.<sup>47-49</sup>

Pityriasis versicolor (tinea versicolor) has a chronic and relapsing nature, requiring re-treatment or prophylaxis.<sup>22,23</sup> Pityriasis versicolor lesions are more extensive in tropical climates,<sup>50</sup> and the microscopic appearance of the organism in skin scales is different from that in temperate climates. Clusters of spherical yeasts with hyphae that may be branched (“spaghetti and meatballs”) are seen in temperate regions, whereas oval or cylindrical yeasts +/- hyphae are seen in tropical regions.<sup>40,50</sup> This suggests that the species of *Malassezia* vary according to geographic location. Data from Spain show that the majority of pityriasis versicolor lesions yielded *M. globosa*, although lesions were often associated with *M. sympodialis*.<sup>17</sup> In Japan, *M. furfur* and *M. sympodialis* are recovered, but not *M. globosa*.<sup>32</sup>

Pityriasis versicolor is usually suspected clinically and the diagnosis is confirmed by examining skin scrapings microscopically for the presence of yeast and hyphae using KOH +/- methylene blue or Calcofluor white. Quantitative polymerase chain reaction (PCR) and DNA sequencing have been used to map the *Malassezia* microflora of both infected and healthy subjects and to provide both species identification and strain typing.<sup>24,32,51-54</sup> Treatment is usually with topical agents: selenium sulfide, ketoconazole, miconazole, and propylene glycol. In severe cases, systemic agents (e.g. oral ketoconazole, itraconazole) may be used.<sup>24,55</sup> Treatment for several months is often necessary.

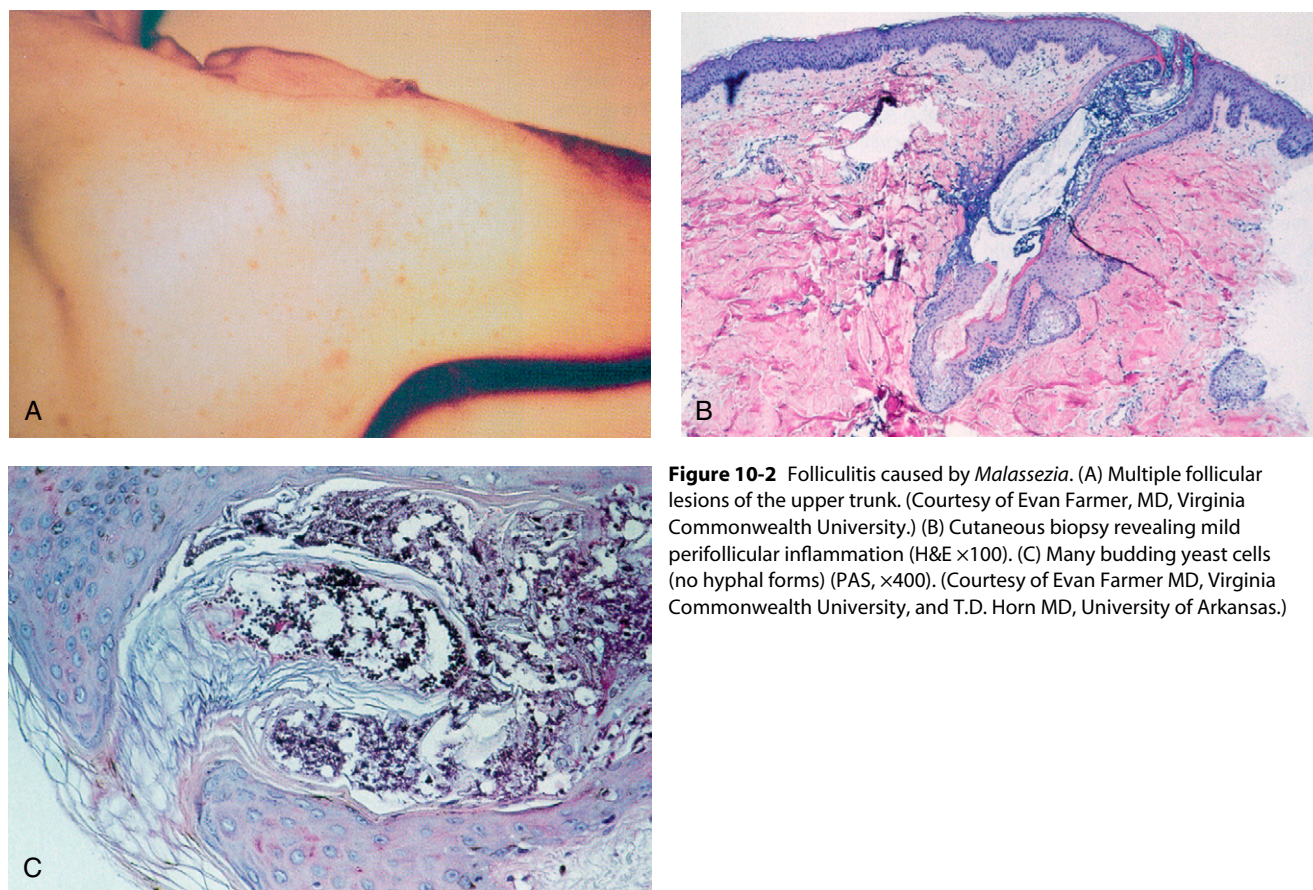
*Malassezia* may also cause a folliculitis (Fig. 10-2) with erythematous, pruritic papules and pustules on the torso, neck, and arms.<sup>56-58</sup> First reported in the setting of antibiotic therapy,<sup>57</sup> it also occurs in patients receiving steroids<sup>59</sup> and in association with pregnancy,<sup>60</sup> leukemia,<sup>61</sup> bone marrow transplants,<sup>62</sup> AIDS,<sup>63</sup> Down’s syndrome,<sup>64</sup> Hodgkin’s disease,<sup>65</sup> diabetes,<sup>66,67</sup> and kidney<sup>68</sup> and heart<sup>69</sup> transplantation. It is common in the tropics,<sup>70,71</sup> most likely due to the heat and humidity. Often it is chronic and may be undiagnosed for years, although in immunosuppressed patients it may spread rapidly and be associated with systemic symptoms.<sup>62,72</sup> Therefore, it is important to distinguish *Malassezia* folliculitis from cutaneous lesions of a hematogenously disseminated infection.<sup>73</sup> Diagnosis of *Malassezia* folliculitis may be made by microscopic examination of superficial scrapings or biopsy. Budding yeasts, rather than the hyphae seen in pityriasis versicolor, are found (see Fig. 10-2).<sup>41,74</sup> Treatment of *Malassezia* folliculitis may be the same topical or systemic antifungal agents used for the treatment of pityriasis versicolor. With either type of therapy, relapses may occur, necessitating long-term or prophylactic therapy.

*Malassezia* may play a role in seborrheic dermatitis, atopic dermatitis, and sebaceous miliaria seen in infants. Evidence for the involvement of *Malassezia* in seborrheic dermatitis stems largely from determinations of population densities of the organism in lesional and non-lesional sites and from descriptions of response to antifungal therapy.<sup>22,23</sup> In one such study, *Malassezia* made up 46% of the microbial flora of the scalp in normal subjects and 83% of the flora in patients with seborrheic dermatitis.<sup>75</sup> Similar results have been reported by others,<sup>76</sup> although this is by no means universal.<sup>77,78</sup> The species of *Malassezia* associated with seborrheic



**Figure 10-1** Case of pityriasis versicolor in an otherwise healthy man. (A) Hypopigmented areas that correlate with areas of pressure from a mail pouch. (B) Histopathology of pityriasis versicolor revealing yeast and short hyphal forms of *Malassezia* confined to the stratum corneum (PAS  $\times 40$ ). (Courtesy of Evan Farmer MD, Virginia Commonwealth University.)





**Figure 10-2** Folliculitis caused by *Malassezia*. (A) Multiple follicular lesions of the upper trunk. (Courtesy of Evan Farmer, MD, Virginia Commonwealth University.) (B) Cutaneous biopsy revealing mild perifollicular inflammation (H&E  $\times 100$ ). (C) Many budding yeast cells (no hyphal forms) (PAS,  $\times 400$ ). (Courtesy of Evan Farmer MD, Virginia Commonwealth University, and T.D. Horn MD, University of Arkansas.)

dermatitis include *M. furfur*, *M. restricta*, *M. globosa* and *M. sympodialis*.<sup>22</sup>

Agents with an anti-*Malassezia* activity decrease the density of the organism and alleviate the clinical condition.<sup>79-84</sup> Although simple overgrowth with *Malassezia* is unlikely to be the sole cause of seborrheic dermatitis, the balance of the evidence suggests that the organism is important in the etiology of this condition and not simply a secondary colonizer.<sup>22</sup>

Atopic dermatitis is a common chronic inflammatory skin disease, the etiology of which remains poorly understood.<sup>22,23</sup> Patients with atopic dermatitis had higher levels of IGE for *Malassezia* when compared to healthy controls.<sup>85,86</sup>

A pathogenic role of *Malassezia* in sebaceous miliaria (neonatal acne, neonatal pustulosis) has also been suggested since *Malassezia* was cultured from 8/13 neonates with erythematous pustulopapules of the face, neck, and scalp.<sup>87</sup> Although antifungals may shorten the duration, spontaneous resolution of lesions may occur, likely related to decreased sebum production.

*Malassezia* has also been associated with a broad range of other superficial conditions, including acne vulgaris,<sup>88,89</sup> dacryocystitis,<sup>90</sup> seborrheic blepharitis,<sup>91</sup> confluent and reticulated papillomatosis,<sup>92-94</sup> onychomycosis,<sup>95,96</sup> nodular hair infection<sup>97</sup> and psoriasis.<sup>94-100</sup> In many of these reports, the isolation of *Malassezia*, and/or response of the condition to antifungals, was taken as proof of its involvement in the

disease, an assumption that is confounded by the organism as a skin commensal.<sup>22</sup>

Opportunistic systemic infections due to *Malassezia* have been described for approximately two decades.<sup>59</sup> The most common systemic infection caused by *Malassezia* is catheter-related fungemia in neonates and other immunocompromised patients.<sup>20,21,26,27,54,101-104</sup> The first report was in a premature neonate who developed fatal pulmonary vasculitis while receiving intravenous lipid for parenteral nutrition.<sup>105</sup> At autopsy, *Malassezia* was found within lipid deposits in the vessel wall of the pulmonary artery and the surrounding lung tissue. Subsequently, a cluster of *M. furfur* pulmonary infections was recognized in three infants hospitalized in a NICU.<sup>106</sup> The organisms were detected by microscopy in lung tissue and were cultured from lung, blood, and other clinical specimens. The infected infants were receiving intravenous lipid. Risk factors included gestational age <26 weeks, hyaline membrane disease, duration of ventilation, duration of antimicrobial therapy, and the presence of a Broviac catheter. This cluster suggested for the first time that *M. furfur* could be transmitted from an infected or colonized infant to other infants.<sup>106</sup> Clinical findings in these cases are indistinguishable from those seen in patients with bloodstream infections of other origins: fever, leukocytosis, thrombocytopenia.<sup>22,23,27</sup> Application of molecular typing methods has documented nosocomial transmission of *Malassezia* spp.<sup>20,54,107</sup> The fungemia and systemic disease reported to date have all been caused by

*M. furfur* or *M. pachydermatis*. Other lipophilic yeasts aside from *M. furfur* may have been involved but were not recognized at that time.

*M. furfur* fungemia has been found in immunocompromised children and adults, all of whom had central venous access devices.<sup>101,108-110</sup> Barber et al<sup>101</sup> called attention to the fact that catheter-related *M. furfur* fungemia may occur in immunocompromised patients, aside from neonates, with central venous access devices whether or not they are receiving intravenous lipids. In addition, peritonitis in patients undergoing continuous ambulatory peritoneal dialysis has been described.<sup>111-114</sup>

*M. pachydermatis* is capable of growing on complex media without lipid supplementation.<sup>102</sup> It has been found to cause otitis media and otitis externa in dogs.<sup>115</sup> Outbreaks of fungemia also have been described in NICU settings, generally involving a single epidemic clone.<sup>20,21,54,107</sup> Notably, unlike the findings in outbreaks caused by *M. furfur*, receipt of lipid infusions and exposure to intravascular devices, other than arterial catheters, have not always been identified as risk factors.<sup>20</sup> Healthcare workers and their pets have been shown to carry a common strain of *M. pachydermatis* and in one NICU outbreak the epidemic clone caused colonization of dogs owned by NICU workers, colonization and infection in the infants, and colonization in NICU healthcare workers.<sup>20</sup>

Because *M. furfur* and the other obligate lipophilic species of *Malassezia* are organisms with unusual growth requirements, communication between physicians and the clinical microbiology laboratory will aid in the diagnosis of bloodstream infections. There are sufficient lipids in a broth-based blood culture system for *Malassezia* to proliferate and be seen by Gram stain but the organisms fail to grow on subculture due to lack of lipids in the agar medium. Laboratory personnel often can suspect *Malassezia* species by their morphology on Gram stain (see biology section) and add lipids to the subculture media at the time of initial detection. Some blood culture systems may not support the growth of *Malassezia* species without exogenous lipid supplementation.<sup>116-118</sup>

All patients with *Malassezia* fungemia should be treated, because prediction of catheter colonization versus true systemic infection is not possible. All classes of antifungal agents with the exception of flucytosine show anti-*Malassezia* activity (Table 10-1).<sup>119</sup> Often, antifungal therapy alone is unsuccessful in eradicating the organism and the indwelling vascular catheter must be removed. Similar to other catheter-related infections, the organism may be embedded in a biofilm with a resultant decrease in the susceptibility to antifungals and protection from host defenses.<sup>34,120,121</sup> Intravenous amphotericin B or fluconazole is the most commonly used antifungal treatment.

## Biology

*Malassezia* spp. are lipophilic yeasts that bud unipolarly, repetitively and enteroblastically, and have a wide attachment between mother cell and emerging bud. Although sexual reproduction has not been discovered for any species, *Malassezia* is considered a basidiomycetous yeast in the class Ustilaginomycetes<sup>122</sup> based on cell wall structure and analysis, staining reaction with DBB, G/C content, and urease positivity.<sup>8</sup>

The 11 species may be distinguished genetically by karyotyping,<sup>123</sup> 26S rRNA sequence,<sup>10,25,124</sup> using PCR,<sup>24,32</sup> and by amplified fragment length polymorphism and sequence analysis.<sup>51</sup> The yeasts can be identified by phenotype using cell morphology and a series of biochemical tests.<sup>10,124</sup> Identification to species is not routinely performed in clinical laboratories, but identification can be made using lipid dependency, utilization/inhibition by Tween 20, 40, 60, and 80 and cremophor (castor oil), reactions with esculin and catalase<sup>8,23,124</sup> and cell morphology studies.

*Malassezia* are distinguishable microscopically from other yeasts by the formation of a prominent monopolar bud scar, or collarette, resulting from the continued formation of daughter cells at the site (Fig. 10-3). Sympodial budding has only been observed with *M. sympodialis*, whereas other species exhibit broad-based or narrow-based monopolar budding. The species also vary from spherical to oval to elongated or cylindrical, have thick cell walls, and range in length from 2 to 6 microns.<sup>22</sup>

The identification of *Malassezia* isolates to species is not useful in the management of skin infection, since the same species can be isolated from healthy skin. However, in the case of fungemia or other systemic infections, recognition of the yeasts as *Malassezia* in the tissue and normally sterile fluids, and identification to species in culture, is often useful.<sup>23</sup> Precise information, often to the subspecies or strain level, is required for the management of appropriate therapy and for investigating the source of the infection.<sup>20,23,54</sup>

## Trichosporon

### Definition

*Trichosporon* is a genus characterized by the production of true hyphae and pseudohyphae, arthroconidia and blastoconidia.<sup>8,125</sup> Prior to 1992 virtually all infections due to this genus were ascribed to *Trichosporon beigeli* (syn. *T. cutaneum*).<sup>7</sup> However, this genus underwent a major taxonomic revision in 1992.

### Epidemiology, clinical characteristics, and treatment

*Trichosporon* spp. are among the most common of the non-*Candida*, non-*Cryptococcus* yeasts isolated from clinical specimens throughout the world (Table 10-2).<sup>9,126</sup> Prior to 1992, *T. beigeli* was the predominant cause of human disease. Partial sequencing of the small and large subunits of the rRNA have clearly delineated numerous species.<sup>7,127,128</sup> These species have different habitats and usually occupy narrow ecologic niches.<sup>7</sup> Some are soil borne and others are associated with animals and humans. Six species are of clinical significance: *T. asahii*, *T. inkin*, *T. mucoides*, *T. cutaneum*, *T. ovoides*, and *T. asteroides*.<sup>7,8,127,128</sup> The first three are regularly isolated from clinical specimens,<sup>128-133</sup> whereas the others are uncommon. Recently, *T. loubieri* and *T. mycotoxinivorans* have been described and reported as causes of disseminated disease.<sup>8,134</sup>

*Trichosporon* was initially recognized as the cause of white piedra, a superficial infection of the hair shaft of the scalp, face, axillary or pubic regions, characterized by soft white,

**Table 10-1** In vitro susceptibilities of non-*Candida*, non-*Cryptococcus* yeasts to new and established antifungal agents

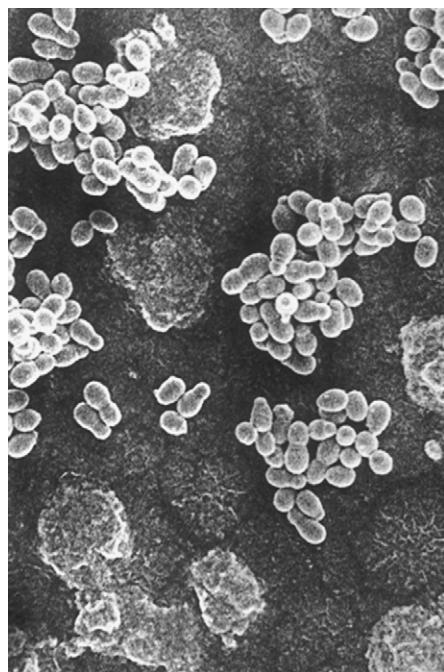
Species	Antifungal agent	No. tested	MIC ( $\mu\text{g/ml}$ )			Reference
			Range	50%	90%	
<i>Malassezia</i> spp.	Fluconazole	70	0.25–4	2	4	119
	Ketoconazole	70	$\leq 0.03$	$\leq 0.03$	$\leq 0.03$	119
	Itraconazole	70	$\leq 0.03$ –0.06	$\leq 0.03$	$\leq 0.03$	119
	Voriconazole	70	$\leq 0.03$ –0.12	$\leq 0.03$	0.06	119
	Albaconazole	70	$\leq 0.06$	$\leq 0.06$	$\leq 0.06$	119
	Flucytosine	70	$\geq 64$	$\geq 64$	$\geq 64$	119
<i>Trichosporon asahii</i>	Amphotericin B	43	1–8	4	4	173
	Fluconazole	43	0.25–16	2	8	173
	Itraconazole	43	0.06–4	0.5	1	173
	Ravuconazole	10	0.25–0.5	0.25	0.5	173
	Posaconazole	24	0.06–>16	0.12	NA	174
	Voriconazole	10	0.25–2	0.5	2	175
	Micafungin	10	>64	>64	>64	176
Non- <i>T. asahii</i>	Amphotericin B	15	0.06–1	0.25	NA	174
	Fluconazole	15	0.5–4	2	NA	174
	Itraconazole	15	0.03–0.5	.012	NA	174
	Ravuconazole	15	0.03–>16	0.5	NA	174
	Posaconazole	15	0.03–0.5	0.12	NA	174
	Voriconazole	15	0.03–0.25	0.06	NA	174
<i>Rhodotorula</i> spp.	Amphotericin B	64	0.5–2	1	1	227
	Flucytosine	64	$\leq 0.06$ –0.5	0.12	0.25	227
	Fluconazole	64	32– $\geq 64$	$\geq 64$	$\geq 64$	227
	Itraconazole	64	0.5–16	2	16	227
	Posaconazole	64	0.5–16	2	4	227
	Ravuconazole	64	$\leq 0.06$ –2	0.25	1	227
	Voriconazole	64	0.25–16	2	4	227
	Caspofungin	64	8–16	16	16	227
	Micafungin	10	>64	>64	>64	212
<i>Sporobolomyces salmonicolor</i>	Amphotericin B	10	1–8	NA	4	183
	Fluconazole	10	64–256	NA	256	176
	Itraconazole	10	0.03–4	NA	0.06	176
	Voriconazole	10	0.06–1	NA	0.12	176
	Ravuconazole	10	0.03–0.12	NA	0.06	176
	Albaconazole	10	0.03–0.12	NA	0.06	176
	Micafungin	10	128	NA	128	176
	Terbinafine	10	0.06–0.12	NA	0.12	176
<i>Saccharomyces cerevisiae</i>	Amphotericin B	74	0.12–2	1	1	249
	Flucytosine	74	0.25–32	0.25	0.25	235
	Fluconazole	74	0.12–16	2	8	235
	Itraconazole	74	0.015–1	0.5	1	235
	Posaconazole	22	0.12–1	0.5	0.5	259
	Voriconazole	17	<0.008–0.5	0.06	0.12	260
	Caspofungin	15	NA	0.5	1	261

(Continued)



**Table 10-1** In vitro susceptibilities of non-*Candida*, non-*Cryptococcus* yeasts to new and established antifungal agents—cont'd

Species	Antifungal agent	No. tested	MIC (µg/ml)			Reference
			Range	50%	90%	
<i>Pichia anomala</i>	Fluconazole	58	2–16	4	8	296
	Itraconazole	58	0.015–1	0.25	0.5	296
	Voriconazole	58	0.03–0.5	0.25	0.25	296
	Amphotericin B	58	0.12–1	0.5	1	296
	Caspofungin	58	0.03–0.25	0.12	0.25	296
<i>P. (Kodomaea) ohmeri</i>	Fluconazole	19	2–32	4	32	276,277,302,306,307
	Itraconazole	17	0.008–0.5	0.125	0.5	276,302,306,307
	Voriconazole	13	0.03–0.5	0.06	0.5	307
	Amphotericin B	19	0.01–1	0.25	0.5	276,277,302,306,307
	Caspofungin	13	0.125–0.25	0.125	0.25	307
	Micafungin	13	0.03–0.06	0.03	0.06	307
<i>Blastoschizomyces capitatus</i>	Fluconazole	23	1–32	8	8	126
	Itraconazole	23	0.03–0.5	0.12	0.25	126
	Posaconazole	25	NA	0.12	0.25	126
	Voriconazole	23	0.03–0.5	0.25	0.25	126
	Amphotericin B	23	0.06–0.25	0.12	0.12	126
	Flucytosine	23	0.12–16	0.12	4	126
	Caspofungin	25	NA	16	>16	261

**Figure 10-3** Scanning electron micrograph of *Malassezia* colonizing the lumen of a central venous catheter. The characteristic collarette at the junction between mother and daughter cell may be seen (Courtesy of S.A. Messer MA, University of Iowa.)

yellow, green or beige nodules composed of hyaline septate hyphae and arthroconidia.<sup>135,136</sup> The disease is worldwide, but occurs more commonly in tropical or subtropical regions. Whereas white piedra was initially considered to be caused by *T. beigellii* (*T. cutaneum*), it is now known that infection of the

**Table 10-2** Geographic variation in the frequency of isolation of non-*candida*, non-*cryptococcus* yeasts from clinical specimens: ARTEMIS surveillance program, 2001–2005<sup>a</sup>

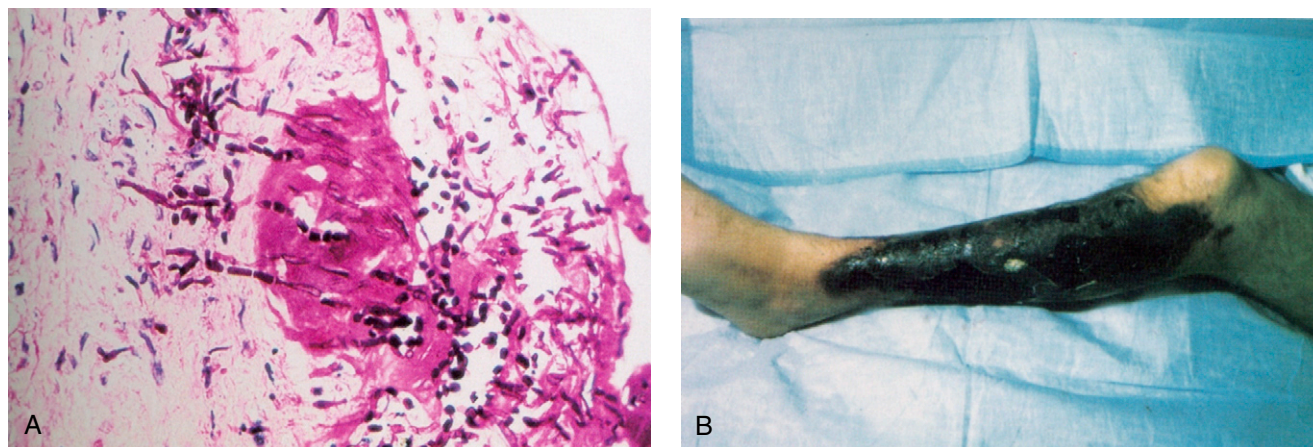
Region	Total no. yeast isolates	% of isolates <sup>b</sup>			
		TSP	RHD	SC	BC
Asia-Pacific	215	41.4	48.8	8.4	1.4
Africa-Middle East	57	49.1	24.6	22.8	3.5
Europe	1,069	29.6	7.1	57.0	6.3
Latin America	278	74.5	13.3	9.0	3.2
North America	156	35.3	34.6	26.9	3.2
Total	1,775	39.2	16.1	39.9	4.8

<sup>a</sup>Data from 124 institutions, compiled from Pfaller et al.<sup>9</sup>

<sup>b</sup>Abbreviations: TSP, *Trichosporon* spp.; RHD, *Rhodotorula* spp.; SC, *Saccharomyces cerevisiae*; BC, *Blastoschizomyces capitatus*.

scalp is caused by *T. ovoides* whereas *T. inkin* infects the genital region. *T. inkin*, *T. asteroides* and *T. cutaneum* have been associated with superficial skin lesions,<sup>137</sup> whereas *T. dermatis*, *T. asahii*, and *T. montevidense* play significant roles in summer-type hypersensitivity pneumonitis.<sup>138</sup>

*Trichosporon* spp. have long been recognized as causing invasive disease in patients who are immunosuppressed in the setting of hematologic or solid organ malignancies or solid organ transplantation.<sup>3,6,130-132,134,139-144</sup> Although



**Figure 10-4** (A) Invasion of liver capsule by *Trichosporon* in a 15-year-old boy with acute leukemia during chemotherapy. Intact hyphae and hyphae breaking into arthroconidia, but no budding cells, are seen (PAS,  $\times 300$ ) (reproduced with permission from Haupt HM, Merz WG, Beschoner WE, Saral R. Colonization and infection with *Trichosporon* species in the immunosuppressed host. *J Infect Dis* 147:199, 1983.) (B) Case of cellulitis caused by *Trichosporon* in a 48-year-old man with chronic lymphocytic leukemia during neutropenia. (Reproduced with permission from Libertin CR, Davies NJ, Holpern J, et al. Invasive disease caused by *Trichosporon beigelii*. *Mayo Clinic Proc* 58:684, 1983. Reprinted by permission of Mayo Clinic Proceedings.)

*T. beigelii* was reported as the cause of invasive infections in the older literature,<sup>142,145,146</sup> *T. asahii* and less frequently *T. mucoides*, *T. inkin*, *T. loubieri*, and *T. mycotoxinivorans* are now recognized as the species causing invasive trichosporonosis.<sup>18,128,131,132,134,147</sup>

*Trichosporon* infections in immunocompromised patients are usually disseminated in nature, involve major organs, and most have been fatal.<sup>3,6,130,142,145,146</sup> Among 43 patients reported in Japan with disseminated disease, 37 (86%) had underlying hematologic malignancy and were profoundly neutropenic.<sup>148</sup> Investigations in a US cancer center have noted a change from disseminated disease to a predominance of catheter-related infection without evidence of tissue invasion.<sup>130,145</sup> The authors speculated that this may be due to the increased utilization of central venous catheters in a susceptible population or that extensive use of fluconazole prophylaxis has been effective in preventing tissue invasion.<sup>130</sup>

Other patients at risk for trichosporonosis include those with AIDS, extensive burns, intravascular catheters, those receiving corticosteroids or undergoing heart valve surgeries.<sup>3,6,130-133,140-142,149</sup> A recent report from three different intensive care units found *T. asahii* causing superficial and invasive disease in six non-granulocytopenic patients.<sup>133</sup> Common risk factors included trauma, prolonged mechanical ventilation, intravascular catheters, and the use of broad-spectrum antimicrobial agents. The authors suggested that the pathogenesis of trichosporonosis in the ICU may be similar to candidiasis with mucosal colonization and subsequent invasion via breaks in mucosal or cutaneous surfaces.<sup>133</sup> Notably, the isolates of *T. asahii* were found to be multiresistant (amphotericin B, flucytosine, azoles, echinocandins) and shared a similar genotype, suggesting a common nosocomial origin.

A colonized gastrointestinal tract and central venous catheters are considered potential portals for entry of this infection. The ability of *T. asahii* to form a biofilm on catheters or biomaterials may be a major factor in persistence of the infection by enhancing resistance to antifungal agents and protection from host defenses.<sup>150</sup> Pulmonary involvement is, however,

the most common site of end-organ disease.<sup>151-153</sup> Chest x-rays show diffuse interstitial infiltrates or patchy reticulonodular involvement.<sup>145,151</sup> Signs and symptoms include dyspnea, cough, and bloody sputum production.<sup>151,152</sup> Organs involved in disseminated infection include the brain,<sup>139,154</sup> eyes,<sup>155</sup> heart,<sup>132,156,157</sup> liver (Fig. 10-4),<sup>158,159</sup> and spleen.<sup>160</sup> Chronic hepatic trichosporonosis mimics hepatic candidiasis and may present on recovery from neutropenia.<sup>161</sup>

Cutaneous findings in hematogenously disseminated *Trichosporon* infection include macules, papules, vesicopustules, and nodules that may be localized to the extremities or found over the entire body.<sup>140,162-165</sup> These lesions may appear similar to those of disseminated candidiasis. Cellulitis may also occur (see Fig. 10-4).<sup>166</sup> Skin biopsy with culture and histology may be necessary for diagnosis.

Disseminated *Trichosporon* infection is often (>70%) diagnosed by blood cultures.<sup>126</sup> Diagnosis may also be made by biopsy of the affected site with culture and histopathologic studies.<sup>167</sup> Yeast forms, arthroconidia (see Fig. 10-4) and hyphal elements that are larger than in *Candida* infections (up to 10 microns) are seen. Another histologic feature is that *Trichosporon* hyphae are often arranged in a radial pattern.<sup>148,167</sup> Although not widely available, immunoperoxidase staining has been used as a diagnostic modality, because discrimination between *Candida* and *Trichosporon* may be difficult using routine fungal stains.<sup>148,167</sup> Of note, the glucuronoxylomannan (GXM) in the cell wall of *Trichosporon* spp. is antigenically and biochemically similar to that of GXM in *C. neoformans*.<sup>168-170</sup> *Trichosporon* may produce high concentrations of GXM during disseminated infections, and it may be detected in serum from infected patients as a cross-reaction in the cryptococcal antigen test.<sup>168-170</sup>

Treatment of trichosporonosis relies on rapid diagnosis and differentiation of *Trichosporon* from *Candida* spp.<sup>6</sup> Susceptibility to amphotericin B is variable (see Table 10-1) and this agent lacks fungicidal activity against *Trichosporon*.<sup>146,171</sup> Clinical failures with amphotericin B, fluconazole and combinations of the two have been reported, and the outcome is generally dismal in the absence of neutrophil

recovery.<sup>6,130,142,145,146,161,171</sup> Kontoyiannis et al<sup>130</sup> have noted that breakthrough trichosporonosis may occur during systemic therapy with most antifungal agents, regardless of the dosage or duration. Most recently, breakthroughs have been in BMT recipients<sup>143</sup> and in patients with hematologic malignancies<sup>144</sup> receiving caspofungin and micafungin, respectively. The multiresistant nature of *T. asahii* makes it a threat for non-neutropenic patients as demonstrated by nosocomial transmission of a strain resistant to amphotericin B, fluconazole, itraconazole, and flucytosine among patients in an intensive care unit.<sup>133</sup> Notably, the new triazoles, voriconazole, posaconazole, and ravuconazole, appear to be more active than fluconazole against *Trichosporon* (see Table 10-1)<sup>129,132,172-176</sup> and voriconazole has been used successfully to treat disseminated *T. asahii* infection in three patients with acute leukemia.<sup>141,147,177</sup>

In vitro susceptibility testing of *Trichosporon* species using Clinical and Laboratory Standards Institute (CLSI) methods generally include only a small number of isolates (see Table 10-1). Arikan and Hascelik<sup>173</sup> reported high amphotericin B minimum inhibitory concentrations (MICs) and moderate susceptibility to fluconazole and itraconazole among isolates of *T. asahii* (see Table 10-1). Rodriguez-Tudela et al,<sup>129</sup> and Serena et al<sup>175,176</sup> documented good activity with the extended-spectrum triazoles. Paphitou et al<sup>174</sup> and Rodriguez-Tudela et al<sup>129</sup> found non-*T. asahii* isolates to be more susceptible than *T. asahii* isolates to amphotericin B, fluconazole, and itraconazole, while the new triazoles gave activity comparable to that seen against *T. asahii* (see Table 10-1). The high MICs reported for the echinocandins, micafungin, caspofungin and anidulafungin indicate that they are unlikely to be effective against *Trichosporon* spp.<sup>175,176,178-180</sup> as evidenced by the reports of breakthrough infection noted previously.<sup>143,144</sup> With limited data, treatment of disseminated trichosporonosis in a persistently neutropenic patient should include voriconazole, catheter removal and administration of granulocyte-macrophage colony-stimulating factor.<sup>6,130</sup>

## Biology

*Trichosporon* is considered a basidiomycetous yeast on the basis of cell wall structure, positive DBB staining, septal pore morphology, urease production, similar RNA sequences, and similarity of a complex polysaccharide antigen (GXM) cross-reactive with the capsular antigen of *C. neoformans*.<sup>181</sup> Gueho et al<sup>127</sup> performed an extensive taxonomic evaluation of 101 strains of *Trichosporon* representing species from humans, animals, and environmental sources. Characteristics used included morphology, ultrastructure morphology, physiologic parameters, ubiquinone systems, G/C content, DNA/DNA reassociation percentages, and partial sequences of 26S rRNA. Subsequent DNA studies by Sugita et al<sup>182,183</sup> confirmed the finding of Gueho et al.<sup>127</sup>

Currently, identification of *Trichosporon* spp. is dependent on cell and colony morphology, biochemical characteristics and temperature studies.<sup>8</sup> This approach is time consuming and not always discriminative. Although the commercial yeast identification method, API 20C AUX (BioMerieux), can identify *T. asahii* and *T. inkin*, it is of little value with other species.<sup>184</sup> Rodriguez-Tudela et al<sup>129</sup> demonstrated the

poor efficacy of biochemical tests to identify seven *Trichosporon* spp. to the species level when compared to sequencing of the intergenic spacer 1 (IGS1) region of the rRNA gene. These authors concluded that sequencing of IGS1, generally only available at reference centers, is mandatory for species identification of *Trichosporon*.<sup>128,129</sup> Correct identification of the species may be significant in making therapeutic decisions in view of their distinct antifungal susceptibility profiles (see Table 10-1).<sup>129</sup> Perhaps it may be more practical to perform antifungal susceptibility testing than identification of *Trichosporon* to species level.<sup>129</sup>

## Rhodotorula and Sporobolomyces

### Definition

*Rhodotorula* and *Sporobolomyces* are basidiomycetous yeast genera that produce carotenoid pigments ranging from yellowish to red that can be visualized with individual colonies. Both genera share physiologic and morphologic properties with *Cryptococcus* spp. *Rhodotorula* spp. recovered from clinical specimens include *R. mucilaginosa* (syn. *R. rubra*), *R. glutinis* and *R. minuta*. *Rhodotorula* spp. can also be recovered from environmental sources and are transient colonizers of moist skin. Among isolates of non-*Candida*, non-*Cryptococcus* yeasts reported in the ARTEMIS surveillance project, *Rhodotorula* was most prominent in the Asia-Pacific region (see Table 10-2). *Sporobolomyces* species are rarely found in clinical specimens; *S. salmonicolor* is the most common species recovered on culture.

### Epidemiology, clinical characteristics, and treatment

*Rhodotorula* species may be isolated from soil, water, fruit juices, milk products, shower curtains and toothbrushes,<sup>8,136</sup> and *Sporobolomyces* species are recovered from various environmental sites. *Rhodotorula* species have been increasingly recognized as important human pathogens.<sup>185-212</sup> Immunocompromised patients, particularly those with central venous catheters or other indwelling devices, are at highest risk for infection, most commonly presenting as fungemia.<sup>185-187,193,194,201,204,205,212</sup> Other sites of infection include endocarditis,<sup>203</sup> meningitis,<sup>189,207</sup> peritonitis,<sup>190,213,214</sup> and ocular infection.<sup>215-217</sup> In contrast, case reports of infections caused by *Sporobolomyces* spp. include one mycetoma in which *S. roseus* was isolated,<sup>218</sup> one patient with dermatitis due to *S. holsaticus*,<sup>219</sup> and several instances of *S. salmonicolor* isolated from clinical specimens. *S. salmonicolor* has been associated with a nasal polyp,<sup>220</sup> lymphadenitis<sup>221</sup> and bone marrow involvement<sup>222</sup> in AIDS patients, a prosthetic cranioplasty infection,<sup>223</sup> a case of endogenous endophthalmitis in a previously healthy woman,<sup>224</sup> and a case of extrinsic allergic alveolitis.<sup>225</sup>

Risk factors for fungemia due to *Rhodotorula* spp. are similar to those described for other opportunistic fungal bloodstream infections.<sup>186,187,194,201,205,212</sup> Recently, prophylaxis or treatment with fluconazole has also been recognized as a risk factor for *Rhodotorula* fungemia.<sup>192,201,204,205</sup> The importance of central venous catheters and fluconazole prophylaxis as risk factors was highlighted in an outbreak of *R. mucilaginosa*



fungemia in a neonatal intensive care unit.<sup>205</sup> Surprisingly, *Rhodotorula* fungemia has been associated with a crude mortality of up to 20%.<sup>201,226</sup>

Standard therapy for infections due to *Rhodotorula* spp. include amphotericin B with or without flucytosine coupled with removal of indwelling catheters, if present.<sup>212,227,228</sup> Given the rarity of clinical infection due to *Sporobolomyces* species, there is clearly no standard therapy. Treatment that has been used successfully includes amphotericin B alone,<sup>223</sup> and amphotericin B followed by either ketoconazole<sup>222</sup> or fluconazole.<sup>221</sup> Most recently, a case of endogenous endophthalmitis was treated successfully with a 2-month course of oral voriconazole (200 mg twice daily).<sup>224</sup> In vitro susceptibility data for a small number of isolates demonstrate resistance to fluconazole and micafungin and very low MICs for itraconazole, the extended-spectrum triazoles, and terbinafine (see Table 10-1).<sup>175,176</sup> The lack of activity by echinocandins against both of these yeasts is important as these agents are often instituted after yeasts are recovered from clinical specimens and before their identification.<sup>212</sup>

## Biology

*Rhodotorula* and *Sporobolomyces* are anamorphic basidiomycetous yeasts of the teleomorphic genera *Rhodospiridium* and *Sporidiobolus*, respectively. *Rhodotorula* is characterized by the production of carotenoid pigments, multilateral budding yeast cells, occasional production of true hyphae or pseudohyphae, inability to assimilate inositol or to ferment sugars, the production of urease, and the elaboration of a capsule or mucoid colonies by some strains. *R. glutinis*, *R. mucilaginosa*, and *R. minuta* can be distinguished by carbohydrate assimilation profiles and utilization of nitrate.<sup>8</sup>

*Sporobolomyces* is an asexual genus of basidiomycetous yeast characterized by the production of carotenoid pigments visualized in colonies from pink to red or orange. Morphologically, true or pseudohyphae are produced, as well as ballistoconidia borne on large sterigmata. All species are urease positive and do not ferment carbohydrates. The species may be homothallic or heterothallic, and teleospore formation can be seen. Three species have been isolated from human infection: *S. roseus*, *S. holsaticus*, and *S. salmonicolor*. The latter two species have the teleomorphs *Sporidiobolus johnsonii* and *Sporidiobolus salmonicolor*, respectively.<sup>8</sup> The most common species, *S. salmonicolor*, can be identified by its carotenoid pigment, distinctive morphology (ballistoconidium formation), urease production, and carbohydrate assimilation patterns.<sup>229</sup>

## Saccharomyces

### Definition

*Saccharomyces* (baker's yeast) is a yeast genus represented by *S. cerevisiae*. It is often associated with fruits, vegetables, and other foods.<sup>136</sup> Humans may become colonized, although this is often transient in nature. Human infections with *S. cerevisiae* do occur,<sup>230,231</sup> and 80% of all cases have been diagnosed since 1990. Approximately 40% (51% of fungemias) were due to *S. cerevisiae* subtype *boulardii*, a probiotic used

for prophylaxis and treatment of diarrheal disorders such as *Clostridium difficile* infection.<sup>230-232</sup>

### Epidemiology, clinical characteristics and treatment

*Saccharomyces cerevisiae* is a colonizer of mucosal surfaces and part of the normal flora of the gastrointestinal tract, the respiratory tract, and the vagina.<sup>234</sup> It is not known, however, whether *S. cerevisiae* is a persistent commensal of the digestive tract or whether it is transiently present after food ingestion. It is isolated from clinical specimens worldwide and accounts for 57% of clinical isolates of non-*Candida*, non-*Cryptococcus* yeasts in Europe but is uncommon in the Asia-Pacific and Latin American regions (8–9%) (see Table 10-2).

Since the 1980s, *S. cerevisiae* has also been isolated from individuals with pathogenic conditions and has been a cause of invasive fungal infections.<sup>230,231,235,236</sup> Clinical syndromes such as pneumonia,<sup>237,238</sup> empyema,<sup>239</sup> liver abscess,<sup>237</sup> peritonitis,<sup>240-242</sup> vaginitis,<sup>243</sup> esophagitis,<sup>238,244</sup> urinary tract infection,<sup>245,246</sup> cellulitis,<sup>247</sup> unexplained fever, and septic shock<sup>239</sup> have been reported. Its presence in sterile sites has been ascribed to rupture of local barriers or to very high fungal loads. Portals of entry include translocation of organisms from the enteral or oral mucosa<sup>248-252</sup> and contamination of intravenous catheter insertion sites.<sup>253</sup>

The most important syndrome caused by *S. cerevisiae* is fungemia. It occurs in immunosuppressed patients and critically ill patients, but also in relatively healthy hosts.<sup>230,231</sup> Population-based studies suggest that *S. cerevisiae* accounts for 0.1–3.6% of all episodes of fungemia<sup>254-256</sup> and the crude mortality rate is 28%.<sup>231</sup>

It is important to note that 80% of the reports of *S. cerevisiae* causing serious invasive fungal infection have been published since 1990 and 40% of those have implicated *S. cerevisiae* subtype *boulardii*.<sup>230,231</sup> Risk factors associated with invasive *Saccharomyces* infections are similar to those reported for invasive candidiasis,<sup>257</sup> except for treatment with a probiotic containing *S. cerevisiae* subtype *boulardii*.<sup>230</sup> Among 37 *S. cerevisiae* subtype *boulardii* infections reported in the literature, 32 (86%) had a history of probiotic therapy.<sup>230</sup> In the five cases in which *S. cerevisiae* subtype *boulardii* was considered to be the etiologic agent, although the patients were not administered a probiotic preparation, the genotype similarity between the patient and the probiotic strain strongly suggested nosocomial acquisition, with catheters being a likely portal of entry.<sup>254,258</sup> When probiotic capsules are opened for administration through a nasogastric tube, viable yeasts can be detected in a 1-meter radius due to aerial dispersion. The organisms may persist on environmental surfaces for up to 2 hours, and can be detected on the hands of healthcare workers even after vigorous hand washing.<sup>231,254</sup> In this setting, it is easy to see how central venous catheters may be the portal of entry.<sup>253</sup>

*Saccharomyces* infection is clinically indistinguishable from invasive candidiasis. Fever is common (75%) and chorioretinitis may occur. Therapy for *S. cerevisiae* fungemia or other invasive forms of infection should be withdrawal of the probiotic regimen, if relevant, administration of an antifungal agent, and removal of central venous catheters.<sup>230,231,253-255</sup>

Although there is no antifungal agent of choice, *S. cerevisiae* has been consistently susceptible to amphotericin B and flucytosine (see Table 10-1).<sup>235,259-261</sup> Most isolates are moderately susceptible to fluconazole and itraconazole (see Table 10-1); however, high-level resistance to fluconazole has been described<sup>234</sup> with therapeutic failures.<sup>262</sup> Although data are scarce, voriconazole, posaconazole and caspofungin have good activity (see Table 10-1). Voriconazole has been used successfully to treat sepsis due to *S. cerevisiae* subtype *boulardii* following initial failure of fluconazole.<sup>262</sup>

Recent reviews of fungemia and invasive fungal infection due to *S. cerevisiae* have concluded that:<sup>230,231,258</sup>

- fungemias can occur in immunocompetent patients and may contribute to morbidity and mortality in immunocompromised patients
- enteral translocation of ingested organisms and central venous catheter hub or insertion site contamination are the main portals into the bloodstream
- prevention of central venous catheter-related fungemias can be accomplished by attention to catheter care and other prophylactic measures
- fluconazole and amphotericin B are effective antifungals; removal of central venous catheters alone has also been effective in some cases.

Finally, Herbrecht and Nivoix<sup>232</sup> have recommended that *S. cerevisiae* subtype *boulardii* probiotic administration should be contraindicated for patients of fragile health and patients with a central venous catheter. The risk–benefit ratio of probiotics in ill patients, given the demonstrated risk of infection, is an important question.

## Biology

*Saccharomyces* is an ascomycetous genus of yeast characterized by round to oval multilateral budding yeast cells and short, rudimentary (occasionally well-developed) pseudohyphae.<sup>8</sup> Ascoconidia are produced by some strains and production can be enhanced by using Fowells' acetate agar for 2–5 days at room temperature. Asci contain 1–4 round, smooth ascocoonidia. The genus is nitrate negative and all species are capable of fermenting carbohydrates.<sup>8,263</sup> *S. cerevisiae* may be identified by carbohydrate assimilation patterns (especially raffinose) and microscopic morphology.

The literature refers to *S. cerevisiae* subtype *boulardii* as *S. boulardii*;<sup>230,231</sup> however, molecular studies have shown this to be an invalid taxon that should be a subtype<sup>264</sup> or a variety<sup>265</sup> of *S. cerevisiae*. Routine biochemical and morphologic characteristics are insufficient to differentiate *S. cerevisiae* subtype *boulardii* from *S. cerevisiae sensu stricto*. Molecular techniques such as ribosomal DNA sequencing, random amplified polymorphic DNA, DNA chromosomal profiles, and mitochondrial DNA restriction analysis have all been used to identify isolates of *Saccharomyces* to the species level.<sup>253-255,258,266,267</sup> These methods have yet to be fully validated for their discriminatory power and ability to discriminate *S. cerevisiae* subtype *boulardii* from *S. cerevisiae*.<sup>230</sup> The length of a particular microsatellite-containing locus reliably distinguishes between the *boulardii* subtype and other *S. cerevisiae* strains.<sup>268</sup>

## Pichia

### Definition

The teleomorphic genus *Pichia* is an ascomycetous yeast found in plants, fruits, soil, and other organic material. Human infections are generally sporadic, but outbreaks have been reported.<sup>269-277</sup> The two species associated with human infection are *P. anomala* (formerly *Hansenula anomala*; anamorph, *Candida pelliculosa*) and *P. (Kodomaea) ohmeri* (anamorph *Candida guilliermondii* var. *membranaefaciens*); *P. anomala* is encountered more often than *P. (Kodomaea) ohmeri*.

### Epidemiology, clinical characteristics, and treatment

*Pichia anomala* is found in soil, plants, and fruit juices and has also been described as a contributor to the microbial flora of the skin, throat and alimentary tract. It is a rare pathogen, but has been recognized as an emerging opportunistic pathogen causing serious infections in immunocompromised patients and in infants in neonatal intensive care units.<sup>269-275</sup> The first report of human infection involving *P. anomala* was in an infant who died of interstitial pneumonia in 1953.<sup>278</sup> Subsequently, *P. anomala* has been implicated as a pathogen in sporadic cases of pneumonia, endocarditis, fungemia, ventriculitis, urinary tract infection, and oral mucosal infection.<sup>279-292</sup>

*Pichia anomala* has been the cause of outbreaks in neonatal, pediatric and adult intensive care units.<sup>269-275,293-295</sup> The most extensive outbreak of *P. anomala* fungemia occurred over 23 months (April 1996 to February 1998) and involved 379 neonates and children (4.2% of all admissions) in a pediatric service in India.<sup>271</sup> Infants with *P. anomala* fungemia had a lower mean birth weight, a younger mean gestational age, and a longer hospital stay than controls without fungemia. Colonization was detected in 28% of neonates admitted to the unit and 20% of them subsequently developed fungemia. Molecular epidemiologic studies suggested a common source. Among neonates with *P. anomala* fungemia, the most common site of colonization was the umbilicus (80%), followed by the mouth (60%), the rectum (30%), and the groin (20%). *P. anomala* was isolated from one postinfusion drip set, a wash basin, and the hands of two healthcare workers. The authors hypothesized that cross-contamination occurred via the hands of the healthcare personnel, with a possible role of the inanimate hospital environment and colonized infants as a reservoir of *P. anomala*.<sup>271</sup>

An additional seven outbreaks of *P. anomala* fungemia, ranging from two to 24 patients, in either neonatal or pediatric intensive care units<sup>269,270,274,275,293-295</sup> and two outbreaks involving adult patients (four and eight patients, respectively)<sup>272,273</sup> have been reported. In several of these outbreaks, analysis of the epidemic curve and molecular epidemiologic markers suggested a common exogenous source for *P. anomala* fungemia. Risk factors include central venous catheters, previous antibiotic therapy, parenteral nutrition, long duration of hospitalization and, in infants, low birth weight and prematurity. Crude mortality rates as high as 41% have been reported,<sup>271,273,274</sup> underscoring the severely compromised nature of the infected patients. These findings support *P. anomala* as an emerging nosocomial pathogen.

Treatment strategies have involved amphotericin B, flucytosine or fluconazole, coupled with removal of the central venous catheter. In the outbreak setting, reinforcement of infection control measures, especially care of central venous catheters, usually controlled the epidemic; however, in two large outbreaks elimination of the organism was only possible with oral nystatin prophylaxis together with topical iodophore at venepuncture sites.<sup>271,295</sup>

Data on the antifungal susceptibility of *P. anomala* are scarce.<sup>270,274,275,281,285,286,295,296</sup> Amphotericin B with or without flucytosine remains the drug of choice for *P. anomala* infections due to its in vitro and in vivo activity (see Table 10-1).<sup>281,297</sup> Fluconazole exhibits modest activity in vitro (see Table 10-1) and has been successful in treating *P. anomala* fungemia;<sup>285</sup> however, fluconazole-resistant *P. anomala* has emerged in immunocompromised patients receiving fluconazole prophylaxis or therapy.<sup>286</sup> Recently, Matta et al<sup>296</sup> demonstrated good activity of voriconazole and caspofungin (see Table 10-1), although they noted that MICs for these new agents were high compared to those for *C. albicans*. There is no clinical experience with voriconazole or caspofungin.

*P. (Kodomaea) ohmeri*, a teleomorphic form of *Candida guilliermondii*, is an environmental yeast used commonly in the food industry for fermentation of pickles, rinds, and fruit. Kurtzman and Fell<sup>298</sup> first isolated this yeast from a clinical specimen of pleural fluid, in which it was thought to be a contaminant, whereas the first documented case of *P. ohmeri* fungemia was reported by Bergman et al<sup>299</sup> in 1998. Subsequently 18 additional cases of fungemia or invasive infection, six of whom died, have been published.<sup>276,277,299-307</sup> Patients had one or more preexisting conditions, such as implants (central venous catheter, dialysis catheter, port-a-cath, pace maker), cancer with chemotherapy with or without neutropenia, prosthetic heart valve, immunosuppression, prematurity, intravenous drug abuse, diabetes, prolonged hospitalization, abdominal surgery, or extensive wounds. Other infections have included endocarditis,<sup>302,304</sup> phlebitis,<sup>306</sup> peritonitis,<sup>301</sup> urinary tract infection,<sup>303</sup> and polymicrobial wound infection.<sup>276</sup> In contrast to *P. anomala*, only one nosocomial outbreak due to *P. ohmeri* has been described.<sup>277</sup>

Most cases of *P. ohmeri* infections have been responsive to removal of catheters and administration of amphotericin B, fluconazole, or itraconazole. All patients who underwent surgical debridement of infected tissue or catheter removal recovered.<sup>276</sup> Currently, there is no clear indication of which antifungal agent is preferable to the others. Isolates appear susceptible to amphotericin B and voriconazole and moderately susceptible to fluconazole and itraconazole (see Table 10-1). MICs are also low for caspofungin and micafungin but clinical experience is lacking.

## Biology

*Pichia* is an ascomycetous yeast genus characterized by multilateral budding, the ability to use nitrates, and the production of hat-shaped ascospores. Both *P. anomala* and *P. ohmeri* can be identified by the production of ascospores and by their typical biochemical profiles. 26S rDNA and internal transcribed spacer (ITS) region sequencing facilitate earlier and more reliable identification than phenotypic methods.<sup>291,320</sup> *P. ohmeri* colonies have a membranous surface and exhibit

a characteristic change in color on CHROMagar from pink to blue over a 48-hour period.<sup>277,303,307</sup>

## Blastoschizomyces

### Definition

*Blastoschizomyces* is a genus with many similarities to *Trichosporon* species. There is only one species – *B. capitatus* (teleomorph, *Dipodascus capitatus*).<sup>308</sup> Former names for this organism were *Trichosporon capitatum*, *Geotrichum capitatum*, and *Blastoschizomyces pseudotrichosporon*. *B. capitatus* is commonly found in the environment and may be recovered from the skin, gastrointestinal tract, and respiratory tract of healthy humans. Invasive disease has been documented in immunocompromised patients.

### Epidemiology, clinical characteristics, and treatment

Although *B. capitatus* may be isolated from environmental sources, the two largest studies of the epidemiology of infection with this organism failed to reveal a common environmental source.<sup>126,309</sup> The experience of Girmenia et al,<sup>126</sup> however, suggests that the geographic distribution of *B. capitatus* is by no means homogenous. In a 20-year multicenter retrospective study coupled with a review of the international literature, these investigators found a significantly higher frequency of *B. capitatus* infections in Europe, which accounted for 87% of reported cases.<sup>126</sup> Furthermore, 87% of the European cases occurred in Italy, Spain, and France, suggesting that climatic factors may play a selective role in the epidemiology of *B. capitatus* infections. These findings are supported by recent data collected during the ARTEMIS DISK Surveillance Study conducted between 1997 and 2005 in more than 30 countries (see Table 10-2).<sup>9</sup> Among the 86 isolates of *B. capitatus* recovered in this survey, 78% came from Europe and 42% of the European isolates were recovered in Italy.

The comprehensive reports of Girmenia et al<sup>126</sup> and Martino et al<sup>309</sup> demonstrate that while opportunistic infections with *B. capitatus* can occur in various types of immunocompromised hosts, those with hematologic malignancies are by far the most common victims of this infection. Among the 88 cases of *B. capitatus* infection reported in the world's literature, 92% had hematologic disease.<sup>126</sup> Most of the infected patients had acute leukemia and had been treated with conventional cytotoxic chemotherapy. Very few had received a blood stem cell transplant and the infections usually occurred during a period of profound neutropenia (neutrophil count, less than 100/mm<sup>3</sup>).

Infection with *B. capitatus* presents similarly to that of invasive candidiasis in neutropenic patients.<sup>126,309</sup> Thus, most (>70%) patients have fungemia, but 60–80% of patients with *B. capitatus* infection develop deep organ involvement, compared with only 10–20% of patients with candidemia.<sup>309,310</sup> The 30-day mortality associated with *B. capitatus* ranges from 60–80%.<sup>126,309</sup> As with *Trichosporon*, a chronic disseminated form of *B. capitatus* infection, similar to chronic disseminated candidiasis, may be seen upon resolution of neutropenia.

Case reports and case studies document widespread solid organ invasion including lung, liver, spleen, kidney, bone,



central nervous system, and heart.<sup>126,309,311-319</sup> Patients with pulmonary involvement present with productive cough, and chest x-ray films show infiltrates (some of which may be mycetomas).<sup>316,320</sup> Focal nodular lesions may be seen by imaging studies in patients with hepatic, renal or splenic involvement, and laboratory assessment invariably shows abnormal liver function tests.<sup>309,316,320</sup> Central nervous system involvement manifests clinically by neurologic deficits and radiographically by the appearance of focal intracerebral lesions.

Diagnosis of *B. capitatus* infection is usually made by culture of blood or other affected sites. Transient colonization with *B. capitatus* may occur, involving stool, urine, and oral mucosal sites.<sup>126</sup> Biopsy may be useful to prove invasive disease (e.g. in patients with pulmonary symptoms and positive cultures, lung biopsy may be used to show histologic evidence of tissue invasion). Notably, Girmenia et al<sup>126</sup> conducted epidemiologic surveys of *B. capitatus* colonization and infection and found that in the absence of other identifiable pathogens, the recovery of *B. capitatus* from respiratory tract specimens of patients with clinically documented pneumonia was indicative of probable pulmonary infection with this organism. Unlike *Trichosporon*, *B. capitatus* is not known to cross-react in the cryptococcal antigen test; however, recent clinical and laboratory data suggest that *B. capitatus* produces a soluble antigen that is cross-reactive with *Aspergillus* galactomannan.<sup>321</sup>

Data on the antifungal susceptibilities of *B. capitatus* are limited (see Table 10-1); however, resistance to fluconazole and decreased susceptibility to amphotericin B have been reported.<sup>312,322,323</sup> In vitro susceptibilities determined by CLSI methods (see Table 10-1) indicate high levels of susceptibility to amphotericin B, itraconazole, posaconazole and voriconazole.<sup>261,323</sup> Most isolates are susceptible to flucytosine and fluconazole, although isolates with decreased susceptibility to both of these agents have been observed.<sup>261,314,323</sup> Caspofungin lacks any meaningful activity against this organism (see Table 10-1).<sup>261</sup>

The optimal approach to therapy of *B. capitatus* infections is not yet defined. Indeed, up to 36% of cases present as breakthrough infections during treatment or prophylaxis with amphotericin B or fluconazole.<sup>126,309,312,314,324</sup> Some investigators believe that *B. capitatus* has decreased susceptibility to amphotericin B;<sup>324,325</sup> however, the strains tested generally appear to be susceptible (see Table 10-1) and recent clinical experience in patients with leukemia was favorable with amphotericin B.<sup>126,309</sup> The excellent activity of voriconazole and posaconazole suggests that they may be useful agents in the treatment of *B. capitatus* infections.<sup>126,326</sup> Rapid removal of central venous catheters, adjuvant immunotherapy, and novel antifungal therapies (e.g., voriconazole, posaconazole, or high-dose fluconazole plus amphotericin B) are recommended for treatment of this rare but devastating infection.<sup>3,6,126,309,312</sup>

## Biology

This genus is characterized microscopically by extensive production of true hyphae, pseudohyphae, annelloconidia resembling arthroconidia, and the lack of blastoconidia. *B. capitatus* is non-fermentative and urease negative. It may be differentiated from *Trichosporon* spp. by specific assimilation patterns, urease negativity, and by the ability to grow on Sabouraud's

glucose agar at 42°C and on cycloheximide-containing agar at room temperature.<sup>8</sup>

## Conclusion

With growing populations of immunocompromised patients, infections due to yeast species that were previously considered to be unusual and/or non-pathogenic are likely to become increasingly common. This diverse group of organisms will pose significant diagnostic and therapeutic challenges.

This chapter was not intended as a compilation of all yeasts that may be pathogens. We have provided descriptions of a number of these species, however, to demonstrate the changing spectrum of fungal disease and the need for communication between clinician and microbiologist in the diagnosis of such infections. Treatment recommendations for these infections are not standardized, given the relative rarity of their occurrence; however, as such infections become more frequent, it is anticipated that additional reports will help clarify the optimal therapeutic regimens.

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# Aspergillus

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## Introduction

*Aspergillus* species are ubiquitous moulds that grow on organic matter. Humans inhale hundreds of conidia per day, usually without adverse consequences except for a small minority of people. The clinical manifestations of aspergillosis are determined by the host immune response to *Aspergillus* species with the spectrum ranging from a local inflammatory response to systemic dissemination. For the clinician, aspergillosis presents a diagnostic and management challenge.

*Aspergillus* is a large genus, containing over 200 species. Only a small number of these species, however, have been associated with disease. Of these, over 95% of all infections are caused by *A. fumigatus*, *A. flavus*, and *A. niger*. Several additional species of clinical importance include *A. nidulans*, *A. terreus*, *A. oryzae*, *A. ustus* and *A. versicolor*. *A. fumigatus* causes most cases of both invasive and non-invasive aspergillosis, and almost exclusively allergic forms of aspergillosis.

Recent monographs and reviews have more than adequately covered the organism.<sup>1-3</sup> In particular, we wish to highlight the Proceedings of the Second Advances Against Aspergillosis Conference, 22–25 February 2006, Athens Greece<sup>4</sup> and the *Aspergillus* website ([www.aspergillus.org.uk](http://www.aspergillus.org.uk)).

## Description of the genus

The fungi classified in the genus *Aspergillus* are anamorphic (asexual) filamentous organisms that reproduce by means of asexual propagules (“spores”) termed “conidia.” Teleomorphic (sexual) forms of many aspergilli have been described. A number of reference sources on the aspergilli culminated in the 1965 manual by Raper and Thom.<sup>1</sup> Since that time numerous new species and varieties have been described.

The identification of species of *Aspergillus* is not easy. Several excellent guides are available to aid in the identification of the common species of medically important aspergilli.<sup>5,6</sup> In identifying aspergilli, it is important to keep in mind that there is variation among strains within a species, as well as among species. Therefore characteristics of the various groups of aspergilli may overlap.

The pathogenic species apparently share some properties not found in other species. One obvious feature is the ability to grow efficiently at 37°C. The production of proteolytic enzymes is another property or virulence mechanism that has been investigated in the pathogenesis of invasive and allergic disease. Isolates from cases of invasive aspergillosis in humans all showed the ability to digest elastin, regardless of the species. Finally, antigens with protease activity have been isolated from *Aspergillus* spp. and have been shown to react with sera from patients with allergic aspergillosis, aspergilloma, or invasive disease.

## Genomics

*Aspergillus fumigatus* strain Af293<sup>7</sup> contains eight chromosomes ranging in size from 1.8 to 4.9 Mb. There are 9,926 predicted protein-coding genes with a mean gene length of 1431 bp. About one-third of these predicted genes (3288) are of unknown function. Additionally, there are at least 12 mitochondrial copies per nuclear genome. Comparison with the genomes of *A. nidulans* and *A. oryzae* revealed ~500 genes unique to *A. fumigatus*, including genes encoding arsenate reductases and additional genes that may have been transferred from soil bacteria by means of a horizontal gene transfer process. Other notable findings include a complete gene complement for heterothallic sex, a cell wall assembly process that is quite different in structural detail from any yeast, at least 28 gene clusters encoding proteins involved in secondary metabolism and mycotoxin production, evidence of numerous cell death pathway components and at least 168 efflux pumps for drugs, toxins, and macromolecules. The sequences of *A. nidulans* and *A. oryzae* were published at the end of 2005,<sup>8</sup> with the sequence of *A. niger* completed but not publicly available. For further information and updates<sup>9,10</sup> see: ‘Aspergillus Genomics’ on the *Aspergillus* website, as above.

*Aspergillus* spp. have been the subject of numerous epidemiologic studies.<sup>10</sup> The most useful typing techniques are DNA-based methods including the random amplified polymorphic DNA technique, microsatellite length polymorphisms, restriction fragment length polymorphism (RFLP) analysis

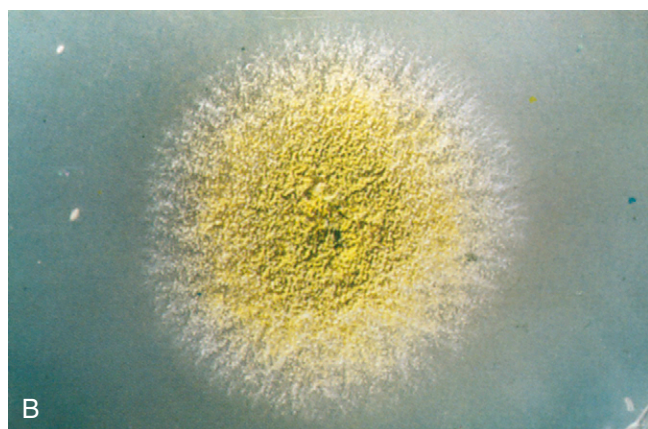
using retrotransposon-like sequences as probes, and multilocus sequence typing. The results of typing clinical isolates indicate that most of the invasive aspergillosis (IA) patients were infected by a single strain. Genetic analysis could not discriminate between clinical and environmental isolates of *A. fumigatus*, indicating that every strain present in the environment is a potential pathogen if it encounters the appropriate host.

Typing studies led to the discovery of a new pathogenic species, *A. lentulus*, and to the identification of several species not known previously to be pathogenic. Typing studies also revealed the existence of two genetically isolated groups within a global *A. fumigatus* population. *A. fumigatus* was found to be the first example of a true cosmopolitan fungus where recombination played an important role in *A. fumigatus* populations.

## Morphology

### Description of colony appearance

Colonies of aspergilli may be black, brown, yellow, red, white, green, or other colors depending on the species and the growth conditions (Fig. 11-1). In addition, the color of the aerial parts of the fungal colonies and the pigmentation of the underlying medium may be different.



**Figure 11-1** (A) Culture of *Aspergillus fumigatus*. (B) Culture of *Aspergillus flavus*.

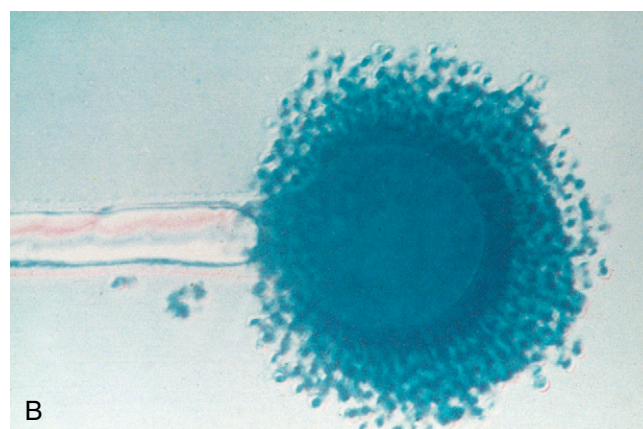
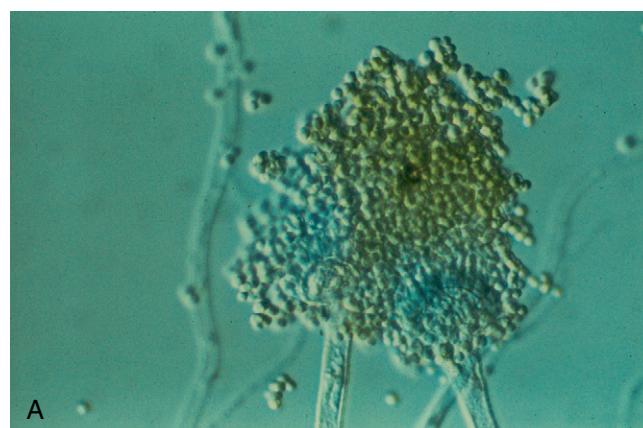
## The mycelium

The mycelium of *Aspergillus* spp. is similar to that of most other fungi. It is well developed, branching, hyaline and septate. The mycelium can produce copious amounts of enzymes, and some produce mycotoxins. The mycelial phase of *Aspergillus* spp. is characterized by vigorous growth and an abundant production of conidia.

The architecture of the *Aspergillus* hyphal wall may have a considerable influence on the response of the fungus to antifungal drugs.<sup>11</sup> Caspofungin is an inhibitor of 1,3- $\beta$ -D glucan synthesis (GS) that produces dramatic morphologic changes in actively growing hyphae. Despite the apparent fungistatic in vitro activity against *Aspergillus* species, compounds in this class have strong efficacy in vivo.

## The conidial head

Conidiation in *Aspergillus* involves many common developmental themes including spatial and temporal regulation of gene expression, specialized cellular differentiation, and intercellular communication. After a certain period of vegetative growth, under appropriate conditions, hyphal cells stop normal growth and begin conidiation by forming conidiophores that bear multiple chains of conidia.<sup>12,13</sup> The conidial head of aspergilli is composed of conidia, phialides, metula if present, the vesicle, and the conidiophore, all of which often arise from a foot cell (Fig. 11-2).



**Figure 11-2** (A) *Aspergillus fumigatus*. (B) *Aspergillus flavus*.

In most species the shape, the size, and the color of the conidial heads within the same colony show no variation.

## The conidium

Conidia are usually globose with a rough surface and occur in various sizes. The length of conidial chains, their density of packing, and their orientation around the conidia heads vary from species to species and are useful for identifying species and group. The individual color of the conidia (which may be hyaline), the collective color of the conidial mass, and the color of the aerial hyphae give the colors observed in the colonies.

Mechanisms controlling asexual sporulation (conidiation) in *A. fumigatus* have now been studied by examining functions of four key regulators: GpaA (Gα), AfflbA (RGS), AffluG, and AfbrlA.<sup>12,13</sup> Expression analyses of GpaA, AfflbA, AffluG, AfbrlA, and AfwetA throughout the life cycle of *A. fumigatus* revealed that, while transcripts of AfflbA and AffluG accumulate constantly, the latter two downstream developmental regulators are specifically expressed during conidiation. Both loss-of-function AfflbA and dominant activating GpaA(Q204L) mutations resulted in reduced conidiation with increased hyphal proliferation, indicating that GpaA signaling activates vegetative growth while inhibiting conidiation.

In invasive and allergic diseases, the surface of *Aspergillus* conidia mediates the first contact with the human immune system following their inhalation. Thus, conidial surface proteins may be reasonable vaccine candidates as well as important allergens. This concept has been explored recently by Asif and colleagues.<sup>14</sup> Twenty six different *A. fumigatus* proteins were identified, 12 of which contained a signal for secretion. Among these were the known major conidial surface protein rodlet A, one acid protease PEP2, one lipase, a putative disulfide isomerase and a putative fructose-1,6-biphosphatase. The known allergen Aspf 3 was identified among the proteins without a signal for secretion. On the basis of the recently annotated *A. fumigatus* genome,<sup>15</sup> proteome analysis confirmed expression of hypothetical proteins and thereby identified additional vaccine candidates and possible new allergens.

Parallel studies have shown that conidia adhere to basal lamina proteins via negatively charged sugars on their surface, presumably sialic acids. Sialic acids are a family of more than 50 substituted derivatives of a 9-carbon monosaccharide, neuraminic acid. Warwas and colleagues have shown that unsubstituted *N*-acetylneuraminic acid is the predominant conidial surface sialic acid.<sup>16</sup> Removal of sialic acids decreased conidial uptake by cultured murine macrophages and type 2 pneumocytes by 33% and 53% respectively. The authors conclude that sialylated molecules on *A. fumigatus* conidia are ligands for both professional and non-professional phagocytes. Krappman and colleagues have reviewed the topic of genetic control of protein synthesis.<sup>17</sup>

## Ecology and transmission

*Aspergillus* exposure and the mechanisms of disease in immunocompetent patients are not well defined for the indoor environment. There are no standardized methods to evaluate contamination with *Aspergillus* in an indoor

environment. While species identification by culture is crucial to investigating building-related infections, the relationship between species-specific cultivable concentrations and respiratory disease is unclear. Detection and quantification of microbiologic markers of *Aspergillus* biomass, such as ergosterol, β1,3 glucans and extracellular polysaccharides, have been shown to be useful methods to assess contamination and to more accurately evaluate microbial exposures in the indoor environment.<sup>18</sup>

*Aspergillus fumigatus* is a ubiquitous fungus that plays an important role in carbon and nitrogen recycling in nature. Because *A. fumigatus* is thermotolerant, it is a predominant organism during the high-temperature phase of the compost cycle. The ability to grow at elevated temperatures and to utilize numerous varied sources of both carbon and nitrogen to support its growth have made *A. fumigatus* an important opportunistic pathogen as well as a vital part of the nutrient-recycling ecosystem.<sup>19</sup> Nutritional versatility has been cited as an important contributor to virulence. Indeed, perturbation of pathways involved with nitrogen or carbon sensing has been shown to reduce virulence in animal models, even when in vitro growth rates have not been altered. Therefore, the remarkable ability of *A. fumigatus* to grow efficiently under a variety of environmental conditions and to utilize a wide variety of substrates to meet its nutritional needs contributes to its role as the predominant mould pathogen of immunocompromised patients.

Growth on plants requires an enzymatic armamentarium that is able to degrade plant cell wall polysaccharides.<sup>20</sup> Indeed, a survey of the *A. fumigatus* genome has shown that it encodes a wide range of glycosylhydrolases that have the capacity to degrade the major plant cell wall polymers.<sup>20</sup> It appears that *A. fumigatus* plays a major role in leaf but not in hardwood degradation. However, the enzymes required to sustain growth on building materials and in dust in indoor environments are not known. *A. fumigatus* is the most frequently found thermophilic fungus. It is able to grow at 55°C and can survive temperatures up to 75°C. Two genes have been directly associated with its thermotolerance.<sup>20</sup>

## Sources of *Aspergillus* in the outdoor and indoor environment

### Outdoor environment

*Aspergillus fumigatus* is one of the most common inhabitants of the airborne fungal flora. A classic study showed that low concentrations of *A. fumigatus* conidia are normally recorded, with a higher incidence during the “winter” months.<sup>21</sup> Counts in the open air and in a hospital ward showed similar fluctuations, the indoor counts being consistently lower. The widespread distribution of decaying leaves following fall represents a potential source of smaller concentrations of conidia but over a much larger area. This availability of decaying plant debris with high water content fulfils the growth requirements of *A. fumigatus* and is the probable explanation of its winter seasonality. A more recent study showed that conidia of *A. fumigatus* were found to be abundantly present in the outdoor air at sites where large-scale composting of sewage sludge was taking place.<sup>22</sup>



### Indoor air

In the hospital setting, construction work may liberate large numbers of conidia which have been associated with outbreaks of invasive aspergillosis. Air treatment systems such as high-efficacy particulate air filtration (HEPA), with or without laminar airflow ventilation, reduce airborne fungal contamination and the frequency of IA. The dynamics of indoor development and distribution is a complex process.<sup>23</sup>

Quantitative polymerase chain reaction (PCR) allows for rapid (2–4 hours), sensitive (as low as one *Aspergillus* conidium) detection and quantification of fungi.<sup>24</sup> During the enlargement of an existing hospital, quantitative PCR was used to monitor *Aspergillus* species populations within the construction site.<sup>25</sup> In a newly constructed operating room, high concentrations of *A. fumigatus* and *A. niger* were found in both floor and surface dust samples. Following cycles of cleaning, the *Aspergillus* contamination was eliminated. In a new neonatal intensive care unit, the floor was found to be contaminated with *Aspergillus* species. Extensive HEPA filter vacuuming achieved nearly non-detectable levels of *Aspergillus*.

Additional studies have consistently shown that fungal concentrations in hospital environments are considerably lower than those in other indoor environments where the air is not filtered.<sup>26–29</sup> These studies demonstrate the effectiveness of air-handling systems and thorough cleaning in reducing fungal contamination.

### Water

Home and hospital water supplies have long been considered to be a potential source of *Aspergillus*. Earlier studies have shown that many fungi can survive in potable water systems, including species of *Aspergillus*.<sup>30</sup> *A. fumigatus* from a hospital water system has been genotypically linked to a case of invasive aspergillosis.<sup>31</sup> However, there is growing evidence that cases of aspergillosis may be acquired outside hospital. Cases diagnosed many months after transplantation suggest that IA is acquired in the community.

Vesper and colleagues at the US Environmental Protection Agency have developed a simple extraction method and rapid (3 hours) quantitative PCR method to specifically detect *A. fumigatus*, *A. flavus*, *A. terreus* and *A. niger* in home tap water and hospital water supplies.<sup>30</sup> Water samples were collected from the kitchen tap of 60 homes.<sup>30</sup> Water samples were also taken from three locations in a hospital. *A. terreus* DNA was found in 16.7% and *A. fumigatus* DNA in 1.7% of kitchen tap samples. None of the *Aspergillus* species were found in the hospital water samples. In a further study of *Aspergillus* in a hospital water system, *A. fumigatus* and *A. terreus* were found in a hospital that uses water storage tanks in the handling of water. Here, *A. fumigatus* and *A. terreus* were found by culture at a rate of 11% and 9% respectively.<sup>31</sup>

### Food

More recent reports have appeared regarding the contamination of food by fungi.<sup>32</sup> In the University Hospital of Grenoble, following three successive invasive aspergillosis cases in a protected environment facility, a food management and surveillance protocol was set up for allogeneic stem cell transplant patients. During a 10-year period, 456 types of food were tested. Filamentous fungi were isolated in 37 of them (8.1%). *A. fumigatus* was found once only in cooked vegetables.

*Penicillium* species were the most frequently isolated mould. This study supports a protocol of careful selection of food types and packaging decontamination.

## Outbreaks

A number of databases were searched systematically for descriptions of *Aspergillus* outbreaks in hospital settings.<sup>27</sup> Fifty-three studies with a total of 458 patients were found. In 356 patients, the lower respiratory tract was the primary site of *Aspergillus* infection. Species identified most often were *A. fumigatus* (154 patients) and *A. flavus* (101 patients). Hematologic malignancies were the predominant underlying disease (299 individuals). The overall fatality rate in these 299 patients (57.6%) was significantly greater than that in patients without severe immunodeficiency (39.4% of 38 individuals). Construction or demolition work was often (49.1%) considered to be the probable or possible source of the outbreak. This review highlights that concentrations of *Aspergillus* species in air below 1 colony-forming unit/m<sup>3</sup> were sufficient to cause infection in high-risk patients. Virtually all outbreaks of nosocomial aspergillosis are attributed to airborne sources, usually construction.

## Monitoring exposure

This should be prospectively performed. Traditional methods include air sampling and analysis of settled dust. Non-culture methods for determining the concentration of fungal biomass in indoor environments include the detection of mycotoxin and fungal cell wall glucans.<sup>33</sup>

## Pathogenicity and virulence

Recent publication of the genomic sequence of *A. fumigatus* signifies enormous progress in aiming at cellular features and gene products that contribute to its pathogenicity. The latest developments in the study of virulence-related characteristics comprise profiling techniques, conditional gene inactivation and precise manipulation of the genome by means of gene targeting. Advances in assessing the virulence potential of particular mutant strains in alternative test systems complement these approaches.<sup>34</sup> Large-scale genome comparisons have shown that no gene sets are shared exclusively by both *A. fumigatus* and any human pathogen sequenced to date, such as *Candida* or *Cryptococcus* species. By contrast, and in agreement with the environmental occurrence of *Aspergillus*, the enzymatic machinery required to colonize plant substrates and building materials has been found in the *A. fumigatus* genome. In addition, the proteome of this fungus contains numerous efflux pumps, including >100 major facilitators that help the fungus to resist either natural aggressive molecules present in the environment or antifungal drugs in humans.<sup>20</sup>

## Role of gliotoxin in the pathogenesis of aspergillosis

Gliotoxin is a mycotoxin with a considerable number of immunosuppressive actions. Comera and colleagues investigated the toxic effects of gliotoxin on human neutrophils at concentrations corresponding to those found in the blood of

patients with invasive aspergillosis.<sup>35</sup> Incubation of the cells for 10 min with 30–100 ng/ml of gliotoxin inhibited phagocytosis of either zymosan or serum-opsonized zymosan without affecting superoxide production or the exocytosis of specific and azurophilic granules. Gliotoxin also induced a significant reorganization of the actin cytoskeleton which collapsed around the nucleus, leading to cell shrinkage and the disappearance of filopodia. This study suggests that gliotoxin can affect circulating neutrophils and favor the dissemination of *A. fumigatus* by inhibiting phagocytosis and the consequent killing of conidia.

### Role of surfactant proteins in the pathogenesis of aspergillosis

Surfactant protein (SP)-D plays an important role in the immune response to *A. fumigatus* in the lungs. Recently, Ooi and colleagues sought to determine whether SP-D is expressed in nasal mucosa in the setting of chronic rhinosinusitis (CRS) with eosinophilic mucus (EMCRS) and investigated the response of SP-D in vitro to fungal allergens.<sup>36</sup> Nasal biopsies from 59 CRS and EMCRS patients, stratified into allergic fungal sinusitis (AFS), non-allergic fungal eosinophilic sinusitis (NAFES), and non-allergic non-fungal eosinophilic sinusitis (NANFES), were studied by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), immunostaining and enzyme-linked immunosorbent assay (ELISA). This study demonstrated the expression of SP-D in both diseased and normal nasal mucosa and that SP-D expression in CRS patients is upregulated by fungal allergens. These results may provide potential novel therapy for the treatment of CRS.

### Invasion of non-phagocytic cells by *Aspergillus*

Many fungi that cause invasive disease invade host epithelial cells during mucosal and respiratory infection, and subsequently invade endothelial cells during hematogenous infection. Most fungi invade these normally non-phagocytic host cells by inducing their own uptake. Angioinvasive fungi, such as *Aspergillus* species, invade endothelial cells from the abluminal surface during the initiation of invasive disease, and subsequently invade the luminal surface of endothelial cells during hematogenous dissemination. Invasion of normally non-phagocytic host cells has different consequences, depending on the type of invading fungus. *A. fumigatus* blocks apoptosis of pulmonary epithelial cells. A recent review discusses the mechanisms by which diverse fungal pathogens, including *A. fumigatus*, invade normally non-phagocytic host cells.<sup>37</sup> *A. fumigatus* conidia and hyphae have been found to induce their own endocytosis by type II pneumocytes. Conidia can also be endocytosed by tracheal epithelial cells. Contact with *A. fumigatus* conidia induces type II pneumocytes to produce pseudopods that engulf the organism. Once the conidia are ingested by the pneumocytes, they traffic to late endosomes/lysosomes.

The endocytosis of *A. fumigatus* conidia by pneumocytes has significant effects on both the organism and the host cell. Germination of the endocytosed conidia is significantly delayed compared with conidia that are grown in the absence of pneumocytes. However, many of the conidia eventually germinate, and the resultant hyphae can escape from the endosome and penetrate the plasma membrane of the pneumocyte. It appears

that this process causes virtually no detectable damage to the host cell.<sup>38</sup>

### Catalases of *A. fumigatus*

The various roles of *A. fumigatus* catalases in the context of aspergillosis have been reviewed recently by Shibuya and colleagues.<sup>39</sup> Since a large body of invasive *Aspergillus* infection occurs as an opportunistic infection in variously impaired defense mechanisms, there is a wide spectrum of histopathologic features of lesions at the site of infection. Accordingly, histopathology of the lesions can be understood as a phenotypical representation of interaction between differently impaired functions of neutrophils and macrophages and virulence factors of invading aspergilli. Consideration of previous pathologic knowledge regarding infection and inflammation provides much important information to predict the pathophysiology of a patient. Meanwhile, detoxification of hydrogen peroxide by catalases has been proposed as a way to overcome this host response. *A. fumigatus* produces three active catalases, one from conidia and two from mycelium. CatAp, a conidium-specific monofunctional catalase, is resistant to heat and metal ions. It appears that conidial catalase is not a virulence factor but that mycelial catalases transiently protect the fungus from the host defense reactions.

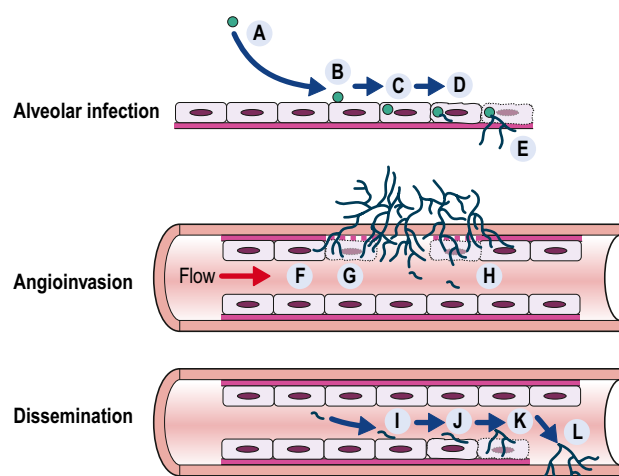
### Internalization of *A. fumigatus* conidia by lung epithelial cells

A number of studies have shown that *Aspergillus* conidia can be internalized by lung epithelial cells and murine macrophages in vitro.<sup>38</sup> A further study was designed to determine the fate of *A. fumigatus* conidia within the endosomal network of these cells.<sup>38</sup> Very few conidia (1–3%) of the initial inoculum survived after 24 hours. However, a small proportion of the internalized conidia survived and had germinated. By 36 hours, the germlings were able to escape the phagosome and form extracellular hyphae without lysis of the host cell.

### Invasion of vascular endothelial cells

Invasion of blood vessels is a key feature of invasive aspergillosis. This angioinvasion contributes to tissue necrosis at the foci of infection and reduces the penetration of leukocytes and antifungal drugs to these sites. There are two types of angioinvasion that occur during invasive aspergillosis.<sup>37</sup> The first type occurs in the lungs (Fig. 11-3). After *A. fumigatus* hyphae have penetrated the pulmonary epithelium, they invade the vasculature by passing from the abluminal to the luminal surface of the pulmonary endothelial cells. This process results in the disruption of the endothelial cell monolayer at the focus of angioinvasion and intravascular thrombosis. This type of angioinvasion is most common. The second type of angioinvasion occurs in profoundly immunocompromised patients. In these patients, hyphae that have entered the bloodstream break off, and the resultant hyphal fragments disseminate hematogenously throughout the body. For these hyphal fragments to invade the target organs, they must penetrate the luminal surface of the vascular endothelial cells.<sup>37</sup>

Kamai and colleagues established in vitro models of luminal and abluminal endothelial cell invasion by *A. fumigatus*.<sup>40</sup>



Invasive aspergillosis occurs via the following steps: infection is initiated by the inhalation of small numbers of airborne conidia (A), which adhere to pulmonary epithelial cells (B), and are readily endocytosed (C). Within the epithelial cells, the conidia germinate to form hyphae (D), which grow by apical extension and escape from the epithelial cells (E). Next, emergent hyphae penetrate the abluminal surface of endothelial cells (F) and induce endothelial cell damage (G). Hyphal fragments disseminate haematogenously (H) and adhere to the luminal endothelial cell surface (I) before invading these cells (J). Luminal invasion results in endothelial cell damage (K) and extravascular invasion of deep organs (L). Steps (A–E) occur in the pulmonary alveoli, (F–H) in the pulmonary blood vessels, and (I–L) in the systemic blood vessels.

**Figure 11-3** Model of *Aspergillus fumigatus* interactions with pulmonary epithelial cells and vascular endothelial cells during angioinvasion and dissemination. (Adapted from Filler and Sheppard <sup>37</sup>).

Luminal invasion by hyphae resulted in both endothelial cell damage and stimulation of tissue factor expression. Abluminal invasion caused less endothelial cell damage than luminal invasion, but greater induction of endothelial cells genes encoding cytokines, leukocyte adhesion molecules and tissue factor. These differences in the endothelial cell response to luminal versus abluminal infection may indicate significant differences in the pathogenesis of hematogenously disseminated versus locally invasive versus aspergillosis.

## Experimental models of invasive pulmonary aspergillosis

Hope and colleagues developed an in vitro model of the human alveoli, consisting of a bilayer of human alveolar epithelial and endothelial cells.<sup>41</sup> *A. fumigatus* penetrated the cellular bilayer 14–16 hours after inoculation. The dynamics of invasion were monitored by determination of *Aspergillus* galactomannan. There was a close temporal relationship between the penetration of the endothelial cell layer and an increase in galactomannan levels. Interestingly, it was shown that galactomannan did not traverse the alveolar–capillary barrier, demonstrating that for diagnostic and therapeutic purposes, the airways and vasculature should be viewed as distinct compartments. When monocyte-derived macrophages were added to the alveolar compartment of the model, there was little suppression of *Aspergillus* growth.

## Immune responses to Aspergillus

### Innate immunity

Following inhalation of conidia, the innate cellular immune system (composed of alveolar macrophages and neutrophils) in the lungs of the immunocompetent host is responsible for killing of the conidia. In immunocompromised hosts some conidia may escape the attack of the innate immune system and establish a systemic infection. It is well established that macrophages represent the first line of defense and ingest and kill *Aspergillus* conidia. To facilitate conidial binding to macrophage membranes,  $\beta$ 1,3 glucan, a major component of the fungal cell wall, is required. On dormant conidia, low amounts of glucan are found but on activated, swollen, germinating conidia high amounts have been detected.<sup>42</sup> Studies like this confirm that glucan is required for conidial adherence and phagocytosis. Furthermore, it appears that Toll-like 2 receptors and the adaptor protein MyD88 are required for efficient conidial phagocytosis.<sup>42</sup> Once phagocytized by alveolar macrophages, conidia are killed by powerful reactive oxygen intermediates (ROIs). If conidial germination occurs before or after phagocytosis, neutrophils are able to kill *Aspergillus* germlings.

### Adaptive immunity

Recent studies have shown that adaptive immunity contributes to the host defense against *Aspergillus* species. In a murine model, mice resistant to infection produced a high level of T helper1 (Th1) cytokines, whereas mice with a high level of T helper2 (Th2) cytokines suffer from disease progression. In a human clinical setting, a study has measured the production of either interferon- $\gamma$  (IFN- $\gamma$ ) or interleukin-10 (IL-10) in culture supernatants of peripheral blood mononuclear cells (PBMCs) stimulated with *Aspergillus* antigens, showing that patients with high IFN- $\gamma$ /IL-10 ratio survive IA.<sup>43</sup>

## Control of the immunopathogenesis of aspergillosis

Dendritic cells (DC) are able to decode the fungus-associated information and translate it into different T helper (Th) and regulatory (Treg) cell responses. The outcome of the Th1/Th2 balance is a critical determinant of the outcome in invasive aspergillosis.<sup>42,44,45</sup> Functionally distinct Treg cells are activated after exposure to *Aspergillus* conidia. Early in infection, inflammation/Th1 reactivity is controlled by Treg cells suppressing polymorphonuclear leukocytes (PMNs) and the immunogenic program of DC. The levels of IFN- $\gamma$  produced in this phase set the subsequent adaptive stage by conditioning the indoleamine 2,3-dioxygenase (IDO)-dependent tolerogenic program of DC and the subsequent activation of tolerogenic Treg cells, which inhibit Th2 cells and prevent allergy to the fungus.

The innate immune system is the first line of defense against *A. fumigatus*. Phagocytes engulf and kill inhaled conidia, but also closely communicate with the adaptive immune system. Recognition of invading microbes is mediated by pattern recognition receptors (PRRs), and Toll-like receptors (TLR) 2 and TLR4 have been implicated in the immune response to *A. fumigatus*. While conflicting data still exist on the relative importance of TLR2 and 4 in recognition of distinct



*A. fumigatus* morphotypes, recent evidence suggests that certain TLR agonists can be used to divert the immune response towards an optimal fungicidal activity in the absence of detrimental inflammatory consequences.

## Interaction of *A. fumigatus* with the alveolar macrophage

In the immunocompetent patient, inhaled conidia are easily cleared by the immune system. Knowledge of the cellular pathways involved in the innate immunity against *A. fumigatus* is poorly represented. Dubourdeau and colleagues investigated the immune response against *A. fumigatus* in murine alveolar macrophages in terms of mitogen-activated protein kinases (MAPK), nuclear factor (NF)  $\kappa$ B and cytokine signaling.<sup>46</sup> Their investigations revealed that in murine alveolar macrophages, MAP kinases, extracellular signal-regulated kinase (ERK) and p38 are activated under in vitro conditions, following addition of *A. fumigatus* conidia. In vivo experiments, however, showed that only ERK is directly involved, because activation of p38 was negligible. Immunosuppression with corticosteroids inhibited phosphorylation of ERK and was directly accompanied by a strongly decreased level of tumor necrosis factor (TNF)- $\alpha$  and additional cytokines. In addition, killing of *A. fumigatus* conidia is reduced using the ERK inhibitor. Therefore, ERK appears to be an essential MAP kinase in the defense against *A. fumigatus*. Activation of the transcription factor NF $\kappa$ B appeared only late after infection, suggesting an association with the intracellular swelling of conidia.

The signaling pathways of the alveolar macrophage involved in the clearance of *A. fumigatus* are poorly understood. In one study, the role of TLRs in the immune response against *A. fumigatus* and their contribution to the signaling events triggered in murine alveolar macrophages upon infection with *A. fumigatus* conidia was investigated.<sup>46</sup> Specifically, the study was designed to examine MAPK and NF $\kappa$ B activation and cytokine signaling. The investigation revealed that immunocompetent TLR2, TLR4, and MyD88 knockout mice were not more susceptible to invasive aspergillosis as compared with wild-type mice and that the in vitro phosphorylation of the MAPKs ERK and p38 was not affected in TLR2, TLR4 or MyD88 knockout mice following stimulation with conidia. In vivo experiments suggest that ERK was an essential MAPK in the defense against *A. fumigatus*, whereas the activation of NF $\kappa$ B appeared to play only a secondary role. The findings demonstrated that TLR2/4 recognition and MyD88 signaling are dispensable for the clearance of *A. fumigatus* under immunocompetent situations.

## Phagocytosis of *Aspergillus* by effector cells

Macrophages are the first line of defense and ingest and kill conidia. C-type lectins represent a family of receptors which recognize pathogen-specific carbohydrates. One of them is  $\beta$ 1,3 glucan, a major component of the fungal cell wall. Luther and colleagues provide evidence that  $\beta$ 1,3 glucan plays an important role for the elimination of *A. fumigatus* conidia.<sup>42</sup> Laminarin, a soluble  $\beta$ 1,3 glucan, and antibodies to dectin-1, a well-known  $\beta$ 1,3 glucan receptor, significantly inhibited conidial phagocytosis.

## Cytokine profiling of pulmonary aspergillosis

Chronic cavitary pulmonary aspergillosis (CCPA) is a slowly destructive form of pulmonary aspergillosis. It has been hypothesized that CCPA cytokine gene polymorphisms would differ from patients with allergic bronchopulmonary aspergillosis (ABPA) and uninfected controls. Sambatakou and colleagues profiled functional cytokine gene polymorphisms for IL-10, IL-15, transforming growth factors (TGF)- $\beta$ 1, TNF- $\alpha$  and IFN- $\gamma$  in patients with CCPA and showed that CCPA may be a consequence of poor control of the inflammatory response in the lung.<sup>47</sup>

## Diagnosis

### Definition of cases and indicators of IA

Establishing the diagnosis of aspergillosis in an immunocompromised patient is difficult because the clinical presentation is non-specific and the fungus is seldom isolated. Interpretation of serologic test results in an immunocompromised patient is difficult because failure to detect precipitins does not rule out aspergillosis, and detection of circulating antigen is not a consistent finding in such patients.

The problems associated with the diagnosis of invasive aspergillosis are highlighted by the diagnostic criteria that have to be fulfilled before patients are eligible for inclusion in clinical trials of new antifungal agents. Often these criteria are either presumptive or definitive, depending on whether tissue diagnosis has been achieved. The following diagnostic criteria have been found to be useful in defining invasive aspergillosis.

- Clinical and radiologic evidence of lower respiratory tract infection: new infiltrates on computed tomography imaging, e.g., halo sign, air crescent sign, or cavity within area of consolidation (major criterion); symptoms of lower respiratory tract infection, e.g., cough, chest pain, hemoptysis, dyspnea, physical finding of pleural rub; any new infiltrate not fulfilling major criterion or pleural effusion (minor criteria).
- Biologic criteria: positive culture or direct microscopic evaluation for *Aspergillus* from sputum, bronchoalveolar fluid samples or sinus aspirate specimen; galactomannan detection in specimens of bronchoalveolar fluid or at least two blood samples.
- Host factors: neutropenia ( $<500$  neutrophils/mm<sup>3</sup> for  $>10$  days); persistent fever for  $>96$  h refractory to appropriate broad-spectrum antibacterial treatment in high-risk patients; body temperature either  $>38^{\circ}\text{C}$  or  $<36^{\circ}\text{C}$  and any of the following predisposing conditions: prolonged neutropenia ( $>10$  days) within the previous 60 days; immunosuppressive regimen within the previous 30 days; history of proven or probable invasive fungal infection; or AIDS.
- Histopathologic criterion: identification of *Aspergillus* hyphae from needle aspiration or biopsy specimen (lung, nose, paranasal sinus, bronchi or sites of dissemination) with evidence of associated tissue damage; or positive culture result for a sample obtained by a sterile procedure from a normally sterile and clinically or radiologically

abnormal site consistent with infection, excluding urine and mucous membranes.

A review of the laboratory diagnosis was recently provided by Hope et al.<sup>48</sup>

## Microscopy and culture

Microscopic examination of sputum preparations is often helpful in the diagnosis of allergic aspergillosis because abundant septate mycelium are usually seen (Fig. 11-4A). Microscopic examination of sputum is seldom helpful in patients with suspected invasive aspergillosis, but examination of bronchoalveolar lavage (BAL) specimens is often rewarding (Fig. 11-4B).

The definitive diagnosis of aspergillosis depends on isolation of the etiologic agent in culture. The fungus may be recovered from sputum specimens from patients with allergic aspergillosis, but cultures from patients with other forms of aspergillosis are less successful. Their isolation from sputum is more convincing if multiple colonies are obtained on a plate or if the same fungus is recovered on more than one occasion. Positive culture may also be a sign of transient exposure to inhaled conidia. If sputum cannot be obtained from an immunocompromised patient with a lung infiltrate, alveolar lavage specimens should be obtained. Isolation of an *Aspergillus* sp. from such specimens is often indicative of infection but is positive in less than 60% of cases. *Aspergillus* spp. may be recovered from sputum or BAL specimens, especially in patients with diffuse pulmonary infiltrates, whereas recovery of fungus from patients with focal lesions is more difficult. A positive culture is not always indicative of infection but may merely represent colonization. It has been estimated that around 40% of neutropenic patients from whom *Aspergillus* spp. are isolated do not have invasive disease. *Aspergillus* spp. are seldom recovered from blood, urine or CSF specimens, although cultures of blood have been positive in occasional patients with endocarditis.

## Serologic tests

Many potential systems for the immunodiagnosics of aspergillosis have been described. Those based on detection of antibody to the organism have been successful in allergic

aspergillosis and aspergilloma, and those used for the detection of fungal antigen have great potential for the diagnosis of invasive aspergillosis.

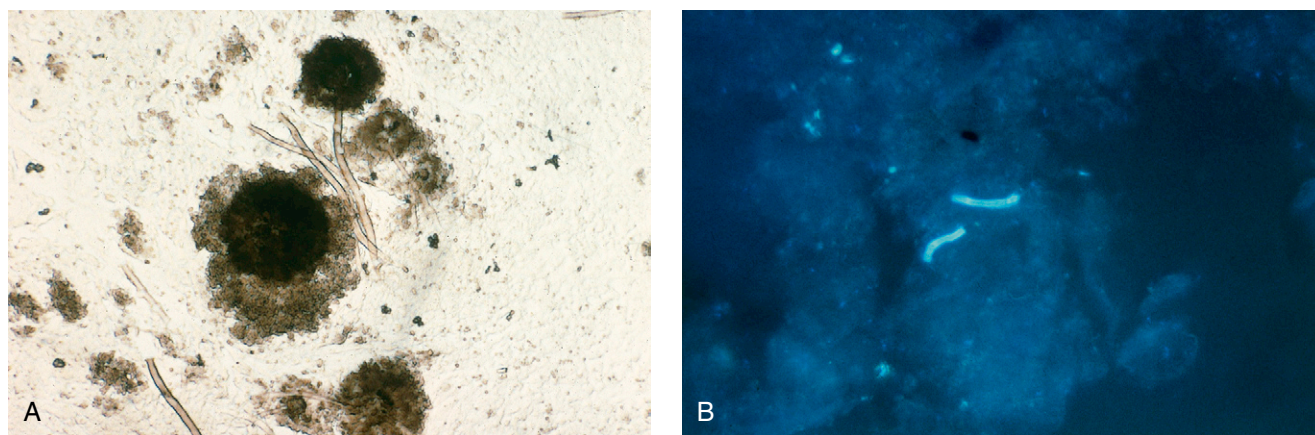
### Antigens of *Aspergillus* spp

The vast heterogeneity of antigen types and the lack of understanding of their relevance in vivo have prevented the development of standardized serologic tests. Most of the antigens identified in crude extracts from *A. fumigatus* grown in vitro lack specificity.

The detectable levels of anti-*Aspergillus* spp. antibodies found in everyone are thought to result from the continuous inhalation of conidia from the atmosphere. Although conidial and mycelial antigens are similar, growth phase-specific antigens have been demonstrated in *Aspergillus* spp. A number of specific antigens of *A. fumigatus* have been tested to quantify the anti-*Aspergillus* antibodies in sera of patients with aspergilloma, ABPA, and IA.<sup>49</sup> In a recent report it was shown that in spite of the variability observed in the immune responses of individual patients, quantification of the antibody titers against the 18 kDa ribonuclease (RNU), the 360 kDa catalase (CAT), and the 88 kDa dipeptidylpeptidase V (DPPV) was useful for the diagnosis of aspergilloma and ABPA.<sup>49</sup> Differential diagnosis of ABPA was even possible among cystic fibrosis as well as non-cystic fibrosis patients. In the group of immunocompromised patients with IA, no antibody response was mounted in response to the *Aspergillus* infection in any of the patients. Interestingly, about half of the patients with proven IA came to the hospital with high titers of anti-*Aspergillus* antibodies, suggesting that they were infected upon entry to the hospital. These results suggest that recombinant RNU, CAT, and DPPV have a great potential in the serodiagnosis of all forms of aspergillosis in the immunocompromised and immunocompetent patient.

### Tests for antibodies to *Aspergillus* spp

Antibody levels have been monitored successfully in a number of cases of IA.<sup>50,51</sup> With these highly sensitive methods for antibody detection, a problem of specificity arises because IgG antibodies to *Aspergillus* antigens can be detected in a proportion of healthy persons. The usefulness of antibody detection in invasive aspergillosis may become clearer now that kits



**Figure 11-4** (A) Direct microscopy appearance of *Aspergillus niger* in sputum from a case of allergic bronchopulmonary aspergillosis. (B) Hyphal fragments of *Aspergillus fumigatus* in sputum stained with Calcofluor white.

are commercially available for individual immunoglobulin classes.

## Detection of *Aspergillus* antigen

*Aspergillus* antigen galactomannan has become an important and reliable tool for the early diagnosis of invasive aspergillosis, as reviewed in Verweij and Mennink-Kersten.<sup>53</sup> The galactomannan molecule, which is detected by the commercial sandwich ELISA (Platelia *Aspergillus*, Biorad), was found not to be a single molecule but a family of molecules that have the epitope that reacts with the monoclonal antibody. The cut-off level is now 0.5 worldwide, which will help to further standardize this tool. Both false-negative and false-positive reactivity is encountered.

In recent years, increased experience has been gained with the Platelia *Aspergillus* enzyme immunoassay. However, the excellent sensitivity and high positive predictive value reported in earlier studies cannot consistently be reproduced. As expected, this stems from major methodologic and clinical heterogeneities between studies. Many reviews highlight the between-study heterogeneities but conclude that the detection of serum galactomannan can be used to define a case of IA in well-defined populations of at-risk patients (for example, Maertens et al 2006<sup>53</sup>).

### Cross-reactivity in the galactomannan (GM) test

A number of filamentous fungi appear to cross-react in the GM ELISA test. Giacchino and colleagues reported three cases of invasive *Geotrichum capitatum* infection in patients with acute leukemia for which an ELISA for *Aspergillus* galactomannan was positive, with no evidence of aspergillosis.<sup>54</sup> Supernatants obtained from suspensions of 17 *G. capitatum* strains gave positive reactions with the *Aspergillus* galactomannan ELISA. These clinical and laboratory data seem to suggest that *G. capitatum* produces a soluble antigen that is cross-reactive with *Aspergillus* galactomannan.

### False positivity in the GM test

Several reports have described a high rate of false-positive *Aspergillus* GM test results for patients treated with piperacillin-tazobactam. In one study, Aubry and colleagues first examined the relationships between intravenous administration of three  $\beta$ -lactam antibiotics and the occurrence of false-positive GM test results in hematology patients.<sup>55</sup> The study examined the kinetics of clearance of GM after the cessation of treatment. Sequential serum samples from 69 patients who had received  $\beta$ -lactams were analyzed by using a Platelia *Aspergillus* test. A significant association was found between GM positivity ( $\geq 0.5$ ) and the administration of  $\beta$ -lactams ( $P < 0.0001$ ). The direct role of  $\beta$ -lactams in patients' serum positivity was assessed by testing 39 batches of  $\beta$ -lactams, of which 27 were positive for GM. None of the latter were positive according to a fungus- and *Aspergillus*-specific PCR. The kinetics of the decrease of GM was analyzed on sequential serum samples obtained after treatment. By use of a non-linear regression model, the average time to negative antigen was assessed to be 5.5 days, with a half-life of elimination of GM of 2.4 days (95% CI, 1.8–3.0). This study confirms that the administration of  $\beta$ -lactams containing GM is responsible for false-positive diagnostic results, even up to 5 days after the cessation of treatment.

Additional factors recognized as causes of false positives in the GM test include antifungals, such as caspofungin,<sup>56</sup> and plasmalyte in BALs.<sup>57</sup>

### Kinetics of galactomannan

Hope and colleagues established an in vitro model of the human alveolus, consisting of a bilayer of human alveolar epithelial and endothelial cells.<sup>41</sup> Galactomannan was used to measure the antifungal effect of macrophages and amphotericin B. It was found that *A. fumigatus* penetrated the cellular bilayer 14–16 h after inoculation. Galactomannan levels were inextricably tied to fungal invasion and were a robust measure of the antifungal effect of macrophages and amphotericin B.

In conclusion, the double sandwich enzyme immunoassay, which detects galactomannan in serum samples, has been available in Europe for over a decade and in the USA since May 2003, for the diagnosis of invasive aspergillosis. However, the availability of the double galactomannan enzyme immunoassay is center variable in the USA and although its analytic performance in the diagnosis of invasive aspergillosis is well documented, its routine use in clinical practice is limited.

### The fungitell test

A highly sensitive (1 pg/ml) colorimetric assay used for the detection of 1,3- $\beta$ -D-glucan (BG), an integral cell wall component in a number of pathogenic yeasts and filamentous fungi but not zygomycetes or *Cryptococcus neoformans*, has been commercially available for some time (Fungitell, Associates of Cape Cod, East Falmouth, MA, USA). Thus far, the sensitivity and specificity rates for this test in limited studies have ranged from 55–100% and 52–100%, respectively, as reviewed by Chamilos and Kontoyiannis.<sup>58</sup> Various authors have reported false-positive test results in patients with concomitant bacterial infections, cirrhosis, patients undergoing hemodialysis, patients following abdominal surgery, and patients receiving antibiotics or chemotherapy with particular agents. It also appears that the BG assay is less sensitive and reproducible and becomes positive later in the course of IA when compared with the GM antigen assay.

## Detection of *Aspergillus* DNA by polymerase chain reaction

The main advantages of the PCR appear to be that it detects low burdens of fungal genetic material. A number of new PCR formats have been developed to detect either individual species or panfungal methods to detect filamentous fungi in general. It has become clear that PCR is a useful tool to aid in the diagnosis of invasive aspergillosis. However, it is essential that an optimal method be agreed upon to allow inclusion in future consensus diagnosis criteria. It should be used in conjunction with other methods (e.g., galactomannan (GM) ELISA and high-resolution computed tomography (HRCT)) to enhance the opportunity for detection of this devastating infection. White and colleagues have reviewed the benefits but mainly the limitations occurring throughout the process of molecular testing.<sup>59</sup> The application of whole-blood PCR formats in a routine laboratory setting has been described at a number of UK centers.<sup>60</sup>

Samples in addition to serum or whole blood have been tested for their suitability for PCR assays. Biopsy of cerebral lesions is often not feasible, and culture of *Aspergillus* spp.



from cerebrospinal fluid (CSF) is frequently negative. So far, there are only a few reports of *Aspergillus* DNA detection in CSF. Hummel and colleagues detected *Aspergillus* DNA in CSF samples by a previously described nested PCR assay.<sup>61</sup> Detection of *Aspergillus* DNA in CSF samples has the potential to improve the diagnosis of cerebral aspergillosis. Another issue that a number of studies have attempted to address is that of DNA extraction methods.<sup>62</sup>

#### Prospective screening for IA by PCR

Halliday and colleagues prospectively evaluated a nested PCR assay to detect *Aspergillus* in blood during 95 febrile neutropenic episodes, in patients with hematologic malignancy and hematopoietic stem cell transplant (HSCT) recipients.<sup>63</sup> PCR results were correlated with the diagnostic classification of the 2002 European Organization for Research and Treatment of Cancer/Mycosis Study Group. When two-positive results were used to define an episode as “PCR positive,” the sensitivity, specificity, positive predictive value and negative predictive value for “proven”/“probable” IA (n = 13) were 100%, 75.4%, 46.4% and 100%, respectively. Consecutive positive results occurred in 61.5% of these 13 episodes. Overall, PCR positivity preceded standard diagnosis by a mean of 14 days and the median time between positive results was shorter than that in other categories of IA. All 13 episodes occurred in the setting of allogeneic HSCT recipients and acute leukemia. If “eligibility” for antifungal therapy were based on two-positive PCR tests, use of empiric treatment could have been reduced by up to 37%. The nested PCR assay is a practical screening test for excluding IA. Patients with consecutive positive results or intermittent-positive results (within 14 days) warrant immediate investigations for IA and the initiation of antifungal therapy.

PCR-based methods are susceptible to cross-contamination, resulting in false positives. Other methodologic problems include the rigid cell wall of *Aspergillus* species (which demands harsh DNA extraction procedures), the very low number of hyphal elements during systemic infection, and *Aspergillus* colonization of the upper airways and sinuses that can contribute to false positives. These problems and the absence of standardized approaches for specimen selection and handling, DNA extraction, DNA target or amplicon detection have led to divergent results. As a consequence, molecular results are not yet recognized as consensual diagnostic criteria for invasive aspergillosis.

#### Detection of *Aspergillus* DNA in tissue specimens

Lau and colleagues developed a panfungal PCR assay that targets the internal transcribed spacer 1 (ITS1) region of the rDNA gene cluster to detect fungal DNA in fresh and formalin-fixed, paraffin-embedded (PE) tissue specimens from patients with culture-proven (n = 38) or solely histologically proven (n = 24) invasive fungal infection (IFI).<sup>64</sup> PCR products were sequenced and compared with sequences in the GenBank database to identify the causal pathogen. The molecular identification was correlated with results from histologic examination and culture. The assay successfully detected and identified the fungal pathogen in 93.6% and 64.3% of culture-proven and solely histologically proven cases of IFI, respectively. A diverse range of fungal genera were identified, including species of *Candida*, *Cryptococcus*,

*Trichosporon*, *Aspergillus*, *Fusarium*, *Scedosporium*, *Exophiala*, *Exserohilum*, *Apophysomyces*, *Actinomyces* and *Rhizopus*. In five specimens, molecular analysis identified a pathogen closely related to that identified by culture. All PCR-negative specimens (n = 10) were PE tissue in which fungal hyphae were visualized. The results support the use of the panfungal PCR assay in combination with conventional laboratory tests for accurate identification of fungi in tissue specimens.

Two semi-nested PCR assays were evaluated by amplifying DNA of zygomycetes and *Aspergillus* spp. from organ biopsies of 21 immunocompromised patients.<sup>65</sup> The PCR assays correctly identified five cases of invasive aspergillosis and six cases of zygomycosis. They showed evidence of double mould infection in two cases. Both assays were negative in five negative controls and in two patients with yeast infections. Sequencing of the PCR products was in accordance with culture results in all culture-positive cases. In six patients without positive cultures but with positive histopathology, sequencing suggested a causative organism. This study indicates that detection of fungal DNA from biopsy specimens allows rapid identification of the causative organism of invasive aspergillosis and zygomycosis.

#### Comparison of PCR with other diagnostic tests

A number of studies have evaluated the diagnostic utility of both the *Aspergillus* GM antigen and the panfungal PCR assay in the diagnosis of IFI in high-risk febrile neutropenic pediatric cancer patients.<sup>66</sup> During a 1-year period in the authors' institution, 91 febrile neutropenic (FN) pediatric cases at high risk for developing IFI while receiving chemotherapy were investigated. These patients were subjected to clinical evaluation, chest CT scan, conventional blood cultures for bacterial and fungal pathogens, *Aspergillus* GM antigen detection and PCR assay utilizing panfungal primers. Of the 91 FN episodes, 15 were proven IFI, whereas 27 cases were either probable (n = 13) or possible IFI (n = 14), and 49 were unlikely to be IFI episodes. Based on positive results for proven/probable IFI and compared to culture results, panfungal PCR showed sensitivity, specificity, positive and negative predictive values of 75%, 92%, 84% and 87%; respectively. *Aspergillus* antigen test showed a sensitivity of 79%, specificity of 61%, positive and negative predictive values of 54% and 83%, respectively. A negative PCR in the proven and probable cases was closely related to previous antifungal therapy for a prior history of IFI. In patients at high risk for IFI, neither the sensitivity nor specificity of the GM test was sufficient. The results of PCR assay were reasonably specific but not very sensitive and had a chance of missing the diagnosis of IFI.

In another study, a case of cerebral aspergillosis was diagnosed by the detection of *Aspergillus flavus*-specific DNA in brain biopsy and serum specimens.<sup>67</sup> The diagnosis was supported by detection of elevated levels of galactomannan and 1,3  $\beta$ -D-glucan in serum specimens. Despite the presence of dichotomously branched septate hyphae in brain biopsy, the culture remained negative. The inability to isolate the organism in culture suggested that combined therapy of AmBisome and caspofungin was fungicidal for the fungus in the brain abscess.

The capabilities of two quantitative PCR assays for detecting pulmonary aspergillosis have been compared in a rabbit model of invasive aspergillosis.<sup>68</sup> Both methodologies were real-time (RT) based and were compared with quantitative cultures and GM antigen detection. The specificity for PCR-based assays, culture, and GM determination was 100%. The sensitivity of the specific PCR assay was 88.9% in lung samples, 66.6% in serum, 55.5% in blood, and 33.3% in brain specimens. The panfungal assay had a sensitivity of 33.3% in lung and serum samples, with brain and blood specimens invariably negative. Otherwise, 100% of the lungs resulted positive for culture, and all serum samples showed a GM index above 1.0 after 2 days of infection. The specific RT-PCR assay is a reliable technique to detect *A. fumigatus* DNA in vivo comparable to cultures and GM determination. The panfungal RT-PCR assay exhibited low sensitivity to diagnose invasive aspergillosis in rabbits which suggests that it may not be suitable for clinical use.

### Reproducibility of PCR assays for IA

Interlaboratory reproduction of these assays is variable, and no consensus has been reached for an optimal method. A United Kingdom–Ireland evaluation of PCR reaction methods for detection of systemic fungal infections is the first multi-center study of PCR methods for the detection of *Aspergillus* (and *Candida* species), utilizing methods currently used in

the UK and Ireland, by distribution and analysis of multiple specimen control panels.<sup>59</sup> The results for the five *Aspergillus* assays were variable, but two methods were superior in that they detected 10<sup>1</sup> conidia.

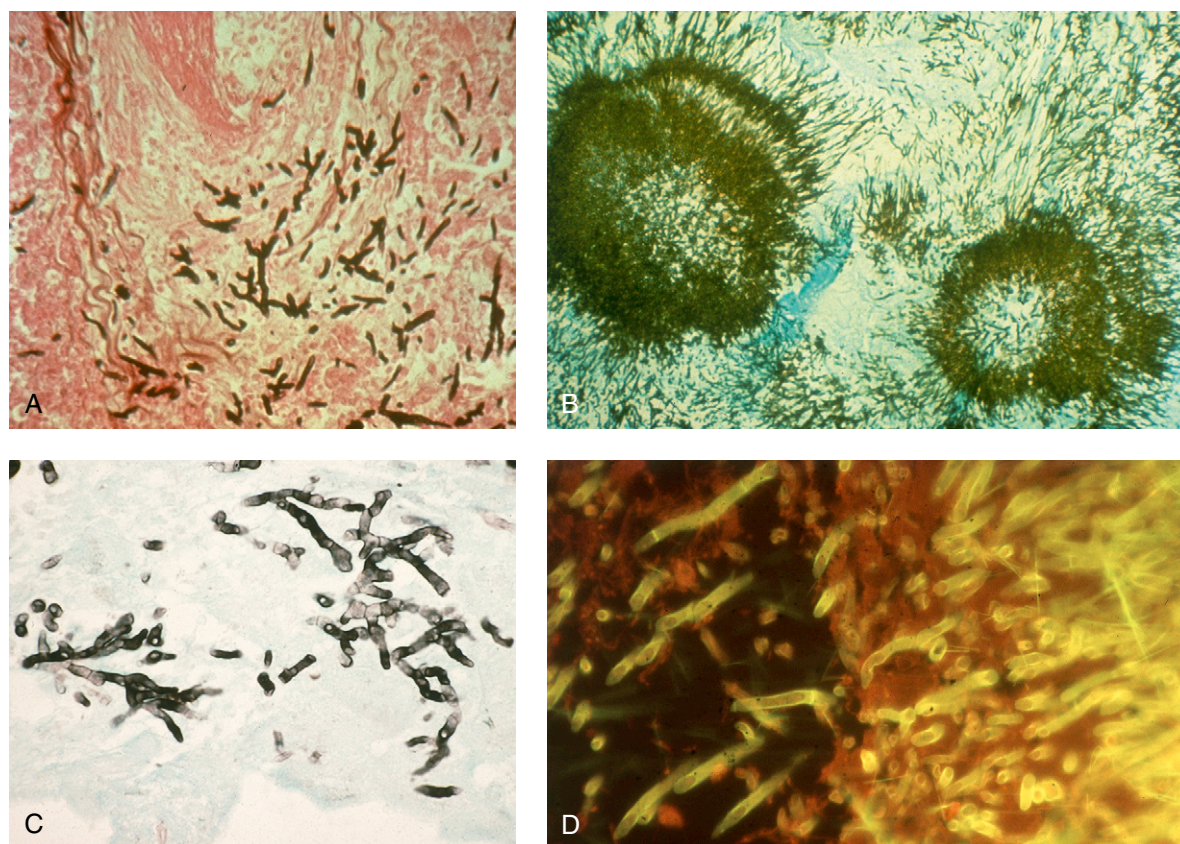
### Histologic features

An extremely important characteristic of the histopathology of invasive aspergillosis is the striking tendency of the fungal hyphae to invade large and small arteries and veins, causing inflammation, thrombosis, and infarction (Fig. 11-5A–C). Frozen sections of biopsy or postmortem material can be stained with Calcofluor white, which will highlight *Aspergillus* hyphae (Fig. 11-5D).

### Prognostic markers in aspergillosis

In allergic forms of aspergillosis, antibody titers may be of some prognostic value. It has been suggested that precipitins may decrease with successful corticosteroid therapy. IgE levels are, however, the most useful parameter to follow in the treatment of ABPA patients. The total IgE often rises before a clinical relapse, and the duration of therapy may be based on the fall in IgE level.

The precipitin test for the detection of circulating *Aspergillus* antigen in blood and urine offers an alternative means of diagnosing aspergillosis in the immunocompromised patient.



**Figure 11-5** (A) Hyphae of *Aspergillus fumigatus* attacking pulmonary parenchyma and blood vessels in a case of invasive aspergillosis. (B) Invasive aspergillosis: invasion of alveoli. (C) Paranasal granuloma in a case of paranasal aspergillosis. (D) Frozen section of lung parenchymal tissue stained with Calcofluor white from a case of invasive aspergillosis showing *A. fumigatus* hyphae.

Changing titers of GM may predict treatment outcomes or indicate the progression of disease. However, *Aspergillus* GM is rapidly cleared from the circulation, and frequent sampling is required for optimal detection of antigen.

Levels of antigenemia and antigenuria may correlate with the clinical course in invasive aspergillosis. Data in animal studies and some from patient series would suggest that antigen levels rise as the clinical condition worsens. Also, some studies have shown that efficacious antifungal therapy decreases antigen levels; however, this has not been confirmed in all studies. At this time it is probably premature to derive any universal correlates for antigen levels in this disease.

## Disease spectrum

*Aspergillus* induces a wide range of clinical syndromes ranging from colonization through to acute invasive disease.<sup>69,70</sup> The allergic manifestations tend to occur in patients with overtly normal immunity. Invasive aspergillosis has been documented in (supposedly) normal hosts, but is highly unusual. It occurs most frequently in the context of HSCT and hematologic malignancy; less common clinical contexts include solid organ transplantation, HIV/AIDS, solid malignancies and chronic granulomatous disease. Historically, neutropenia has been the dominant risk factor for invasive aspergillosis.<sup>71,72</sup> More recently, IA has been increasingly observed in patients with graft-versus-host disease (GvHD) and in non-classic settings, such as within critically ill patients.<sup>73-76</sup>

In profoundly immunocompromised hosts, IA is a rapidly progressive life-threatening infection characterized by extensive hyphal proliferation, angioinvasion and ischemic necrosis. In hosts with more subtle immunologic defects the clinical course is usually somewhat slower, with fewer hyphae, a pyogranulomatous inflammatory infiltrate and inflammatory necrosis.<sup>70,77</sup>

## Allergic bronchopulmonary aspergillosis (ABPA)

ABPA results from an allergic reaction to *A. fumigatus* and is characterized by recurrent fever, cough, wheezing, production of sputum plugs containing *Aspergillus*, and recurrent pulmonary infiltrates. ABPA is observed in 1–2% of patients with asthma and 2–15% of patients with cystic fibrosis.<sup>78</sup> Mucus plugging of the airways may lead to distal atelectasis. ABPA varies in severity, is usually episodic in nature and may progress to irreversible lung damage, which is characterized by central bronchiectasis and upper lobe fibrosis.

The major clinical and laboratory criteria for a diagnosis of ABPA are as follows: (1) asthma, (2) peripheral eosinophilia, (3) pulmonary infiltrates which may be transient or fixed, and (4) immediate skin test reactivity to *Aspergillus*, (5) precipitating antibodies to *Aspergillus* (IgG), (6) elevated serum IgE. Minor criteria include: (1) growth or visualization of *Aspergillus* spp. in sputum, (2) mucus plugs with degenerate eosinophils, (3) infiltrates in CXR suggesting bronchial inflammation. The radiologic findings depend on the stage of disease and range from transient pulmonary infiltrates with hilar or paratracheal lymphadenopathy, through to volume loss, upper zone fibrosis and central bronchiectasis in more advanced cases.

ABPA may also occur in patients with cystic fibrosis and is suggested by the following constellation of clinical, laboratory and radiologic features: (1) clinical deterioration not attributable to other causes, (2) immediate cutaneous reactivity to *Aspergillus* or presence of *Aspergillus*-specific IgE, (3) serum IgE >500 IU/ml and (4) one of precipitins to *Aspergillus*, new or recent bronchiectasis or chest x-ray abnormalities (i.e., mucus plugging or infiltrates which do not clear after the administration of antibacterial agents and physiotherapy).<sup>79</sup>

## Infection of the paranasal sinuses

The *Aspergillus*-related sinus syndromes include: allergic sinusitis, sinus aspergilloma, chronic granulomatous sinusitis (CGD), chronic invasive sinusitis and acute invasive sinusitis.<sup>80,81</sup>

Sinus aspergilloma is the sinus equivalent of pulmonary and renal aspergilloma; there is no evidence of tissue invasion or progressive tissue damage and there are no underlying systemic immunologic defects. The formation of precipitating *Aspergillus* antibodies is characteristic. Sinus aspergilloma develop in areas of structural or functional abnormalities within the sinus. Root canal filling material has been frequently implicated in the pathogenesis, and there may be a connection with heavy metals which may facilitate fungal growth. The fungal ball consists of a dense conglomerate of hyphae arranged in concentric circles. There is no evidence of invasion of the sinuses, although pressure necrosis of the sinus walls may occur.<sup>70</sup>

Chronic granulomatous sinusitis (also called primary paranasal *Aspergillus* granuloma of the Sudan) is a slowly progressive invasive syndrome characterized by florid granulomatous inflammation. Clinically, this syndrome mimics a tumor. Invasion of the cavernous sinus, orbit and brain is frequently seen, and erosion of bone is common. The ethmoid sinuses are invariably involved and a pansinusitis subsequently develops in a majority of patients. This syndrome appears to be restricted to northern Africa, Middle East and the Indian subcontinent. *A. flavus* is the causative pathogen in 90% of cases.<sup>82-86</sup>

Chronic invasive sinusitis is a slowly progressive syndrome which occurs in patients with relatively subtle defects in immunity (e.g., HIV/AIDS, low-dose corticosteroid use, diabetes). The disease is insidious in onset, with evidence of progressive tissue infiltration and destruction over the course of months. Direct extension into contiguous structures is relatively common and the orbital apex syndrome is particularly characteristic.

Acute invasive sinusitis occurs in profoundly immunocompromised patients<sup>87</sup> and is characterized by a rapidly destructive pansinusitis and spread to contiguous structures such as the orbit, brain and carotid artery. The clinical signs and symptoms include facial pain, nasal discharge, epistaxis, and the presence of an anesthetic eschar within the nasal turbinates or palate. Histologically, there is evidence of angioinvasion and coagulative necrosis.<sup>88</sup> A diagnosis is secured by the demonstration of hyphae invading sinus tissue, or inferred from the isolation of *Aspergillus* from a nasal swab. In circumstances in which there are no microbiologic data from the affected site, a positive galactomannan in the blood of a patient with clinical and/or radiologic evidence of sinus disease can be used to establish the diagnosis.<sup>89</sup>



## Aspergillosis of the lower respiratory tract

The lower respiratory tract can be implicated in >90% of cases of invasive aspergillosis.<sup>90,91</sup> The pathologic and clinical manifestations depend on the underlying host and range from saprophytic disease (aspergilloma) through to acute and fulminant invasive disease.

### Aspergilloma

Aspergilloma (or fungal balls) represent a non-invasive (saprophytic) form of aspergillosis. A fungal ball consists of a rounded conglomerate of hyphae, mucus, debris and hyphae which forms within a preexisting pulmonary cavity and is contained within a thickened fibrotic wall (Fig. 11-6A).<sup>70</sup> There is often thickening of the adjacent pleura. Aspergilloma arise most frequently in the context of previous tuberculosis<sup>92</sup> although a range of other pulmonary diseases characterized by cavitation have been described.<sup>93-95</sup> Patients are most often asymptomatic, but may present with chronic cough, malaise, and weight loss. Hemoptysis occurs in 50–80% of cases, and may be massive and life threatening.<sup>96</sup> Chest x-rays reveal a characteristic oval or round mass often with a radiolucent overlying crescent of air (Fig. 11-6B). Pericavitary thickening may be apparent and the fungal ball is frequently mobile.

### Chronic pulmonary aspergillosis

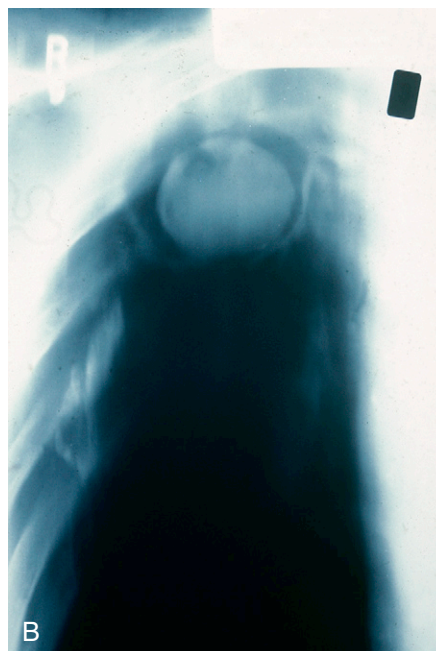
The chronic forms of pulmonary aspergillosis are characterized by slowly progressive cavitary lung disease, with or without fungal balls, chronic respiratory symptoms and precipitating antibodies to *Aspergillus* spp. A number of terms have been applied to this entity; the term “chronic necrotizing pulmonary aspergillosis” is frequently used. More recently,

an attempt to reclassify these conditions has been made.<sup>97</sup> Chronic pulmonary aspergillosis tends to occur in middle-aged patients with underlying or preexisting structural lung disease.<sup>97</sup> Defects (mostly subtle) in systemic immunity also appear to be critical to the pathogenesis: diabetes, corticosteroid use, alcohol abuse appear to be particularly common and abnormalities in mannose binding lectin have also been described.<sup>97,98</sup> Involvement of the pleura is suggestive of a bronchopleural fistula. Extrapulmonary extension or dissemination does not occur.

The clinical signs and symptoms are insidious and occur over a period of months to years. Fever, cough, hemoptysis, malaise, and weight loss are especially common. Typically the radiologic abnormalities progress very slowly (months to years) which mandates repeated imaging and serial comparison of films. Progressive cavitation and the extent of pericavitary thickening are markers of disease activity and can be used to guide antifungal therapy. On occasions massive fibrosis may be seen.<sup>97</sup>

### Invasive bronchial aspergillosis

This entity refers to involvement of the larger (bronchi) accessible airways. There is a plethora of terms and no standardized terminology. The term “*Aspergillus* tracheobronchitis” probably should be reserved for cases of inflammation of the bronchial mucosa without mucosal breach or ulceration. Pseudomembranous tracheobronchitis refers to cases in which there is necrosis and sloughing of the bronchial mucosa, with the mucosal defect obscured by a pseudomembrane. This has been described in a wide range of immunocompromised hosts. An additional term, ulcerative tracheobronchial aspergillosis, which refers to discrete areas of ulceration, was originally described in lung transplantation with involvement of the anastomotic site.



**Figure 11-6** (A) Gross pathology of a resected aspergilloma. (B) Chest x-ray appearance of an aspergilloma within an old tuberculous cavity. Notice the fungal ball free within the cavity.

## Invasive pulmonary aspergillosis (IPA)

The pathology and clinical manifestations of invasive pulmonary aspergillosis are dependent on the underlying immunologic status of the host, and range from acute fulminant disease in patients with profound immunosuppression (Fig. 11-7) through to more insidious presentations in patients with more subtle immunologic defects.

In neutropenic hosts, the histopathologic hallmark of invasive pulmonary aspergillosis is angioinvasion with hemorrhagic infarction.<sup>77</sup> Angioinvasion of large proximal pulmonary vessels may lead to distal wedge-shaped areas of hemorrhagic pulmonary infarction as well as a circumscribed nodule with a central necrotic core, surrounded by a hemorrhagic rim of congested parenchyma (the pathologic correlate of the halo sign; see below). In contrast, non-neutropenic patients exhibit a pyogranulomatous inflammatory infiltrate with inflammatory necrosis; angioinvasion is rare or absent.<sup>77</sup> Cavitation within areas of consolidation is frequently observed in non-neutropenic patients. Patients with CGD exhibit florid non-caseating granulomatous inflammation in which hyphae are relatively scant.

The incidence of hematogenous dissemination from the lungs is difficult to estimate, but may occur in approximately 30% of patients. Less commonly, direct extension to mediastinal structures (heart, great vessel and esophagus) and the chest wall may occur.

There are no pathognomonic signs and symptoms of IPA. Fever, pleuritic chest pain, cough, dyspnea and hemoptysis may all be seen, but none is specific. Physical examination may reveal evidence of focal respiratory signs, but also may be normal. A classic clinical presentation is sudden onset of fever with an associated pleural friction rub which is suggestive of proximal vascular thrombosis and distal hemorrhagic infarction.

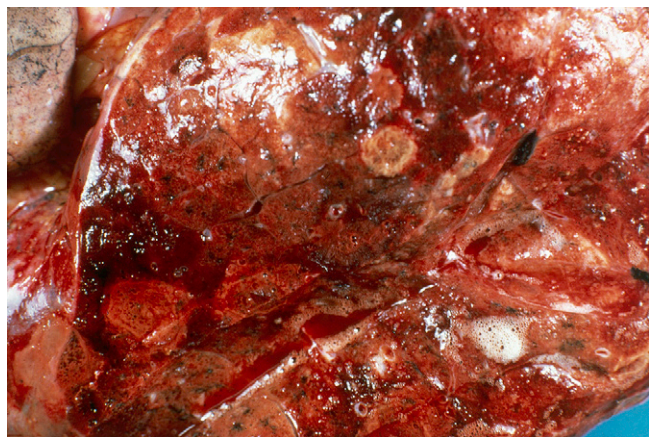
The radiologic manifestations of acute IPA are protean and include a bronchopneumonia, solitary or multiple nodules of varying sizes, lobar consolidation, wedge-shaped infarction and pleural involvement.<sup>99</sup> Routine CT scanning in high-risk patients is more sensitive than plain chest x-ray, and represents an important strategy to establish an early diagnosis in immunocompromised patients (Fig. 11-8).<sup>100</sup> There

are a number of characteristic radiologic signs of IPA in neutropenic patients: (1) a triangular area of infarction, with the base abutting the pleura (resulting from proximal vascular thrombosis); (2) the halo sign, which refers to a macronodule ( $\geq 1$  cm in diameter) surrounded by a perimeter of ground-glass opacity<sup>101</sup> (due to hemorrhagic infarction around a nodule); and (3) an air-crescent sign, which refers to a pulmonary nodule with an associated air-cap which has formed following contraction of a mycotic sequestrum. While these signs are suggestive, they are not 100% specific and can be caused by a variety of infectious and non-infectious processes. In non-neutropenic hosts, cavitating pneumonia is especially characteristic.

A definitive diagnosis of invasive pulmonary aspergillosis requires the demonstration of hyphae invading lung tissue with concomitant culture of *Aspergillus* at the infected site.<sup>89</sup> The recovery of *Aspergillus* from a BAL specimen in a profoundly immunocompromised patient is highly suggestive of IPA. Unfortunately, however, the sensitivity of this procedure is only 30–50%.<sup>48</sup> Routine CT scanning of the chest enables an early (preclinical) diagnosis of IPA.<sup>32</sup> The diagnostic utility of GM and PCR from blood and bronchoalveolar fluid has been exhaustively investigated. In a profoundly immunocompromised host with a pulmonary infiltrate, a diagnosis of IPA is strongly suggested by the detection of galactomannan in BAL or serum.<sup>89</sup> While quantitative PCR in BAL fluid and blood appears promising, difficulties in standardizing molecular assays have hampered its broad applicability in clinical microbiology laboratories.<sup>48</sup> Similarly, the detection of fungal DNA in blood remains problematic, and is yet to be widely embraced as a diagnostic strategy.

## Central nervous system aspergillosis

There are a number of distinct central nervous system (CNS) invasive syndromes.<sup>70</sup> In profoundly neutropenic patients, hematogenous dissemination may occur from a primary site (almost invariably the lungs). Emboli may cause cerebral ischemia and infarction. Vascular invasion by hyphae may lead to hemorrhagic transformation of infarcted areas, or sub-arachnoid hemorrhage.<sup>103,104</sup> In patients with less profound immunologic suppression, cerebral abscess formation may



**Figure 11-7** Gross pathology of lung abscesses in a patient with invasive aspergillosis.



**Figure 11-8** CT scan of a bone marrow transplant recipient showing a large cavitating lesion.



result from hematogenous dissemination. Abscess formation may involve any part of the brain. CNS aspergillosis may also result from direct spread from the contiguous structures, most notably the sinuses.<sup>105</sup> Rarely, CNS aspergillosis may result from direct inoculation at the time of neurosurgery, or following penetrating injury.

The signs and symptoms of cerebral aspergillosis primarily reflect the location of CNS involvement. Confusion, behavioral alterations, reduced levels of consciousness; focal neurologic signs and seizures may be observed and in the first instance should prompt either a contrasted CT or an MRI scan.

The definitive diagnosis of CNS aspergillosis in a neutropenic patient is problematic since a cerebral biopsy is generally precluded. A positive GM or PCR in blood, in the setting of otherwise compatible clinical and radiologic findings, may suggest the diagnosis. The CSF findings, if abnormal, are generally non-specific; a pleocytosis, with a raised protein concentration and a normal glucose concentration, may be observed. The recovery of *Aspergillus* from the CSF is extremely uncommon. An increasing number of reports suggest that the detection of galactomannan and/or DNA in CSF may be a useful means to establish a diagnosis of CNS aspergillosis.<sup>106-108</sup> The diagnosis of CNS *Aspergillus* abscess is relatively straightforward – since drainage is frequently required for therapeutic purposes, there is an opportunity to acquire the relevant diagnostic samples.

## Aspergillus fungemia

*Aspergillus* fungemia is documented infrequently, despite the fact that viable fungal elements must circulate in the blood to cause disseminated disease.<sup>109</sup> False-positive blood cultures may result from laboratory contamination; consequently, the correct interpretation depends on the clinical context. The recovery of *Aspergillus* from the blood of a profoundly immunocompromised patient or in the context of an otherwise compatible illness should never be dismissed without careful consideration. The optimal conditions for the recovery of *Aspergillus* spp. from blood cultures are not established.<sup>109</sup>

## Ocular infections

All components of the eye may be involved by *Aspergillus*.<sup>70</sup> Ocular aspergillosis may arise via hematogenous seeding, extension from the sinuses or direct inoculation. Endogenous endophthalmitis is a manifestation of disseminated disease, and is usually seen in immunocompromised hosts or in the context of intravenous drug use (IVDU). Endogenous endophthalmitis usually presents as sudden visual loss. Invasion of the retina results in necrosis, hemorrhage and eventual detachment. There may be secondary abscess formation and a vitritis. *Aspergillus* scleritis may arise from either direct inoculation or hematogenous seeding. *Aspergillus* keratitis may complicate abrasions or other causes of compromised corneal integrity, including ocular surgery, trauma and contact lens use. *Aspergillus* keratitis may result in corneal perforation (Fig. 11-9). Orbital aspergillosis invariably develops following extension from the sinuses. The orbital apical syndrome (triad of optic neuropathy, proptosis, and ophthalmoplegia) is especially common; in 25% of cases the infection spreads into the brain and is fatal.

## Endocarditis and myocarditis

*Aspergillus* spp. may affect both native and prosthetic heart valves.<sup>70</sup> Native valve *Aspergillus* endocarditis is predominantly observed in patients with profound immunologic impairment<sup>110</sup> but has been documented following coronary artery bypass surgery. *Aspergillus* endocarditis is a well-recognized cause of culture-negative endocarditis. Vegetations tend to be large and friable with a propensity for embolization.<sup>110</sup> Mural endocarditis is highly characteristic and occurs in approximately one-third of those with *Aspergillus* endocarditis.<sup>111</sup> *Aspergillus* infection may also complicate prosthetic valves or other intracardiac devices; the likely pathogenesis is the inoculation of conidia at the time of surgery. Myocardial involvement with abscess formation or mural vegetations may occur as a result of hematogenous dissemination. Myocarditis has been reported in approximately 15% of patients with disseminated aspergillosis, and may result in non-specific electrocardiogram abnormalities and congestive heart failure. Pericarditis is an additional manifestation of cardiovascular aspergillosis.<sup>112</sup>

## Osteomyelitis

*Aspergillus* osteomyelitis may arise from direct inoculation (traumatic or iatrogenic), direct spread from a contiguous focus or from hematogenous dissemination.<sup>70</sup> Children with CGD seem to be at particular risk of rib involvement from direct extension of a pulmonary focus. In addition, there are well-documented cases of direct posterior extension from infected prosthetic aortic grafts to vertebral bodies. Hematogenous dissemination resulting in *Aspergillus* diskitis, vertebral osteomyelitis and epidural abscess formation has been observed in a wide range of patients, including those with hematologic malignancy, IVDU and CGD.

## Otomycosis

Otomycosis refers to the growth of *Aspergillus* spp. (usually *A. niger* or *A. fumigatus*) within the external auditory canal (Fig. 11-10). Patients present with impaired hearing, itching, pain or discharge. Otoscopy reveals greenish or black fuzzy growth on the cerumen or debris in the auditory canal. The course is chronic with acute episodes, especially in summer,



**Figure 11-9** *Aspergillus* keratitis showing a corneal ulcer.



and intermittent remissions. With antifungal treatment the prognosis is good. *Aspergillus* spp. may invade the external auditory canal of immunocompromised patients, extending into contiguous bone or even the brain.

## Cutaneous aspergillosis

Cutaneous aspergillosis can be classified as primary or secondary: primary cutaneous aspergillosis refers to cases following direct inoculation, whereas secondary infection results from hematogenous dissemination.<sup>70</sup> Primary cutaneous aspergillosis classically occurs at catheter insertion sites, and has been documented in neonates and in patients with HIV/AIDS and prolonged neutropenia. The appearance of the lesions is variable, but they are usually described as erythematous to violaceous, edematous, indurated plaques that evolve into necrotic ulcers covered with a black eschar.

In about 5% of patients with aspergillosis, hematogenous spread of infection gives rise to cutaneous lesions. These may be single or multiple, well-circumscribed, maculopapular lesions that become pustular and subsequently evolve into ulcers with distinct borders covered by a black eschar.

## Renal aspergillosis

The renal tract can be affected by *Aspergillus* spp. in a number of different ways.<sup>70</sup> The renal vasculature and parenchyma may be involved as a component of disseminated disease, leading to renal infarction, ischemic necrosis, papillary necrosis and abscess formation. Involvement of the kidneys is usually clinically silent. Hematuria and pyuria may be observed, but cultures are invariably negative. A relatively uncommon manifestation of renal aspergillosis is *Aspergillus* bezoar, which refers to a fungal ball within the renal collecting system. This entity is analogous to pulmonary and sinus aspergilloma and usually occurs in the setting of a preexisting structural abnormality.

## Infections of the gastrointestinal tract

Gastrointestinal tract infection has been detected in 40–50% of patients dying with disseminated infection. The esophagus is the site most frequently involved, but intestinal ulcers also

occur and these often result in bleeding or perforation. Emboli or direct involvement of the mesenteric vessels may result in ischemic necrosis of the gut.

Hepatosplenic infection has been seen in up to 30% of patients with disseminated aspergillosis. The symptoms include liver tenderness, abdominal pain and jaundice, but many patients are asymptomatic. CT scans will reveal numerous small, radiolucent lesions scattered throughout the liver. Modest elevations in alkaline phosphatase or bilirubin concentrations are characteristically seen.

## Aspergillosis in the critical care setting

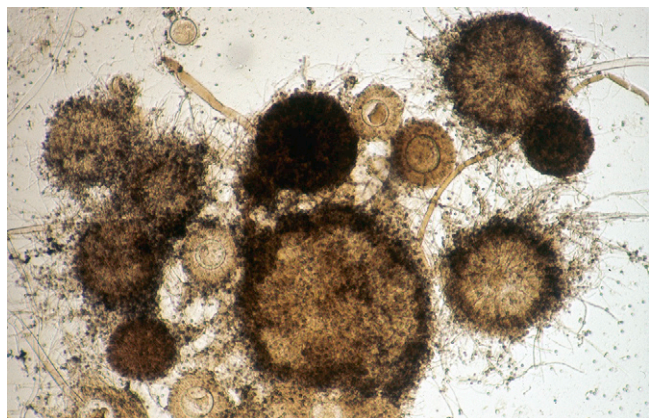
Recent reports have suggested a rising incidence of pulmonary aspergillosis in intensive care unit (ICU) patients.<sup>113</sup> A number of studies have determined the clinical significance of isolating *Aspergillus* from respiratory samples of critically ill patients. In one study from a large cancer center,<sup>113</sup> the case records of 104 were reviewed of all patients over a 6-year period admitted to the ICU, in whom *Aspergillus* was isolated from respiratory samples or lung tissue. *Aspergillus* was isolated for a mean of 6.6 days after ICU admission. Thirty-three percent of patients had hematologic malignancy, 10% had absolute neutropenia, 14% had bone marrow transplant, 11% had HIV infection, and 22% had chronic obstructive pulmonary disease. Upon admission to the ICU, 79%, 43%, and 19% were on antibiotics, corticosteroids or immunosuppressive therapy, respectively. Ninety percent of patients required mechanical ventilation. The mean Acute Physiologic and Chronic Health Evaluation II score on ICU admission was 20.6, with predicted mortality of 35.5%. However, the actual ICU mortality rate for the cohort was 50%. Twenty-eight percent of patients were diagnosed with probable or definite invasive pulmonary aspergillosis, and 72% had *Aspergillus* colonization. On univariate analysis, the significant clinical differences between the two groups were the presence of neutropenia ( $P < 0.05$ ), immunosuppressants ( $P < 0.05$ ), antibiotics ( $P < 0.05$ ) or bone marrow transplant ( $P < 0.05$ ). The differences in Acute Physiologic and Chronic Health Evaluation II score, the need for mechanical ventilation, ICU length of stay, and ICU mortality were not statistically significant. On multivariate analysis, the following factors were independently associated with invasive diseases: bone marrow transplantation ( $P < 0.01$ ), hematologic malignancy ( $P = 0.02$ ), and broad-spectrum antibiotics ( $P = 0.02$ ).

The conclusion from this study was that the isolation of *Aspergillus* in critically ill patients is a poor prognostic marker and is associated with high mortality irrespective of invasion or colonization. Those who are neutropenic, on immunosuppressive therapy, on broad-spectrum antibiotics or had bone marrow transplantation were more likely to have invasive pulmonary aspergillosis.

## Treatment and prevention

### Polyenes

Amphotericin B deoxycholate, until recently, was the drug of choice for invasive aspergillosis. Amphotericin B binds to ergosterol and induces pores within membranes, which are thought



**Figure 11-10** *Aspergillus niger* seen in aural debris from a case of otomycosis.

to be aggregates of drug and ergosterol. Amphotericin B is rapidly fungicidal; its principal limitation, however, is toxicity. The development of renal failure has been associated with an increased length of stay, higher hospital costs and higher rates of mortality.<sup>114-116</sup> In the last decade, dosages of 0.6–1 mg/kg have been generally used; a dose of 0.6 mg/kg has been chosen for the empiric therapy of neutropenic patients with prolonged fever unresponsive to broad-spectrum antimicrobial agents,<sup>117</sup> while a dose of 1 mg/kg is generally considered appropriate for the treatment of established invasive aspergillosis.<sup>118</sup> Importantly, however, there are no definitive clinical data to support any of these choices. The use of amphotericin B for the treatment of invasive aspergillosis has largely been supplanted by safer and more efficacious compounds.

#### Lipid preparations of amphotericin B

In an attempt to circumvent the unwanted effects of amphotericin B, lipid formulations have been developed. The carriers consist of biodegradable lipid molecules which have hydrophobic tails and hydrophilic heads which coalesce in water to form soluble spherical vesicles (liposomes), layered structures or dispersions. This feature offers the opportunity to administer larger dosages while minimizing unwanted side effects.

The serum pharmacokinetics and patterns of tissue distribution of all the amphotericin B preparations (including deoxycholate) are markedly different. There are no data suggesting that any of the lipid preparations have superior efficacy for the treatment of invasive aspergillosis when compared with amphotericin B deoxycholate, although a large body of literature suggests that the lipid preparations are associated with less infusion toxicity and nephrotoxicity than amphotericin B deoxycholate.

Liposomal amphotericin B has demonstrated efficacy for the treatment of established IA in children and adults. Dosages as high as 15 mg/kg/day have been administered without significant side effects.<sup>119</sup> The optimal dosage of liposomal amphotericin B for patients with invasive aspergillosis has not been established. A relatively small EORTC trial suggested that the antifungal response to a dosage of 1 versus 4 mg/kg was comparable<sup>120</sup> and a recently completed trial<sup>164</sup> suggests that the outcome of 3 versus 10 mg/kg is comparable. A dosage of 3–5 mg/kg is recommended by most authorities.

Amphotericin B lipid complex, (ABLC) consists of amphotericin B complexed with two lipids, dimyristoylphosphatidylcholine and dimyristoylphosphatidylglycerol, to produce a ribbon-like structure in a 1:1 drug:lipid molar ratio. ABLC has been compared with liposomal amphotericin for the treatment of unresolved fever in persistently neutropenic patients.<sup>121</sup> While the overall efficacy appears similar, ABLC appears to be associated with more nephrotoxicity and infusion toxicity when compared with liposomal amphotericin B. ABLC has demonstrated efficacy in the treatment of invasive aspergillosis in both adults and children<sup>122-124</sup> but has not been studied in randomized clinical trials. A dose of 5 mg/kg is most commonly used.

Amphotericin B colloidal dispersion (ABCD) is formed from equimolar amounts of amphotericin B and cholesterol sulfate; it has a disk-like form with a mean diameter of 122 nm. ABCD is not as widely used as liposomal amphotericin B and ABLC. A clinical trial demonstrated that ABCD was equally efficacious as amphotericin B for the treatment of invasive aspergillosis.<sup>125</sup>

## Triazoles

The triazoles itraconazole, voriconazole and posaconazole have potent anti-*Aspergillus* activity and have revolutionized the treatment of this syndrome. The triazoles inhibit cytochrome P450 enzymes, which are a superfamily of hemoproteins expressed throughout the phylogenetic spectrum. In fungi, these proteins are important in the biosynthesis of sterols, which are vital components of fungal membranes. The putative drug target for the azoles is the protein 14- $\alpha$  demethylase, which is involved in the removal of methyl groups from lanosterol required for the biosynthesis of ergosterol.

Itraconazole was the first of the triazoles with anti-*Aspergillus* activity to be developed. It is available as a capsule, solution or an IV formulation.<sup>126</sup> Itraconazole is barely soluble at physiologic pH; absorption of itraconazole capsules requires an acidic pH. In critically ill patients with achlorhydria, the absorption of itraconazole may be suboptimal; the resultant low systemic drug exposure may lead to a suboptimal therapeutic response.<sup>127</sup> To circumvent this limitation, a  $\beta$ -hydroxy-propyl-cyclodextrin formulation has been developed, and is available as an IV and oral preparation (itraconazole suspension). The metabolism of itraconazole results in a metabolite, hydroxyl-itraconazole which has a spectrum of antifungal activity identical to the parent compound.<sup>126</sup> Itraconazole levels can be measured by bioassay or HPLC, although the results are not directly comparable (HPLC only measures itraconazole while a bioassay will measure the combined effect of itraconazole and hydroxyl-itraconazole). Itraconazole has a half-life of approximately 24 hours, is lipophilic, and is metabolized via cytochrome 3A4, which has important implications for drug interactions. A trough concentration of at least 0.5 mg/l (determined using HPLC) has been correlated with efficacy<sup>127</sup> but a more complete understanding of concentration-effect relationships is lacking.

Voriconazole is a second-generation triazole which is structurally related to fluconazole. The drug is active against *Aspergillus* species and is used in the treatment of invasive aspergillosis.<sup>128</sup> Voriconazole can be administered orally or IV. Bioavailability is  $\geq 90\%$ .<sup>129</sup> The IV preparation is formulated with a cyclodextrin, which may accumulate in patients with renal impairment. Voriconazole is metabolized via the enzymes CYP 3A4, 2C9 and 2C19. Polymorphisms within these enzymes may result in delayed clearance and accumulation of drug. Voriconazole achieves therapeutic concentrations in the CSF and eye, and also appears to achieve intracellular concentrations.

Voriconazole has a number of clinically important drug interactions: the administration of voriconazole is contraindicated with rifampicin, rifabutin, sirolimus, carbamazepine, ritonavir, efavirenz, quinidine and cisapride and care should be exercised with co-administration with tacrolimus, cyclosporine, phenytoin, HMG CoA reductase inhibitors, warfarin and calcium channel blockers.<sup>129</sup> Voriconazole exhibits linear pharmacokinetics in children<sup>130</sup> but in adults dosage escalation may result in a disproportionate rise in systemic drug exposure. Children require a higher weight-based dosage than adults to ensure equivalent drug exposure.<sup>130</sup>

Side effects include transient visual symptoms, hepatotoxicity, and photosensitivity.<sup>129</sup> There is no consensus regarding the measurement of drug levels to guide the optimization of

therapeutic outcome or prevention of toxicity, although there appears to be a relationship between serum concentrations and both effect and toxicity.<sup>131,132</sup>

Posaconazole is the latest triazole in development, and is structurally related to itraconazole. Currently, the drug is only available as an oral formulation although an IV formulation is being developed. Posaconazole has a large volume of distribution, is highly protein bound and has an elimination half-life of 15–35 hours. Since posaconazole is metabolized via CYP450 enzymes, drug interactions which are similar to those observed with itraconazole and voriconazole are apparent.<sup>133</sup>

## Echinocandins

The three echinocandins caspofungin, micafungin and anidulafungin demonstrate in vitro and in vivo activity against *Aspergillus* spp. The common mode of action is the non-competitive inhibition of 1,3- $\beta$ -glucan synthase, the enzyme involved in the synthesis of 1,3- $\beta$ -glucan.<sup>134</sup> The echinocandins do not exhibit fungicidal activity against *Aspergillus* spp. but rather induce profound morphologic changes: hyphae become short and excessively branched. Histologic studies suggest that drug-exposed organisms have a reduced propensity for angioinvasion, which results in reduced pulmonary injury in experimental invasive pulmonary aspergillosis.<sup>134</sup>

The echinocandins are large water-soluble molecules, which exhibit linear pharmacokinetics.<sup>134</sup> They can be used safely in patients with organ dysfunction, exhibit few significant drug interactions and are associated with a relative paucity of serious adverse effects.<sup>134</sup> Caspofungin and micafungin have an established role for the prophylaxis of invasive fungal infection in high-risk patients,<sup>136,137</sup> and caspofungin may be used as salvage treatment of invasive aspergillosis.<sup>138</sup> The echinocandins may have a unique role in combination regimens, especially with triazoles,<sup>139</sup> although randomized trials to address this concept are pending. The role of the echinocandins as first-line agents for the treatment of invasive aspergillosis remains undefined.

## Surgery

Surgery may have a pivotal role in the treatment of certain forms of invasive aspergillosis, such as osteomyelitis and endocarditis. Drainage of *Aspergillus* abscesses may be appropriate and debridement of skin and sinus lesions may be required to ensure a successful therapeutic outcome. There is occasionally an indication for surgery in cases of invasive pulmonary aspergillosis: single nodular lesions in a patient who requires further immunosuppression may be best treated surgically, and lesions which are near vital structures, such as the heart and great vessels, should also be considered for surgical resection.

## In vivo effects of colony-stimulating factors on neutrophil function and IA

Colony-stimulating factors (G-CSF, GM-CSF) reduce the duration and incidence of neutropenic fevers. In some studies these have decreased the incidence of fungal infections and early survival, although these findings have not been widely replicated.<sup>140,141</sup> Colony-stimulating factors may enhance the activity of antifungal agents.<sup>142,143</sup> There is a renewed interest

in granulocyte transfusions and these should be considered in cases of prolonged neutropenia which is refractory to conventional therapeutic modalities. Interferon  $\gamma$  (IFN- $\gamma$ ) augments the antifungal efficacy of immunologic effector cells, such as macrophages and neutrophils, and this agent has an established role for primary and secondary prophylaxis in patients with CGD.<sup>144,145</sup>

A large number of other innate immune effector molecules may have a future role in the treatment of invasive aspergillosis, including the pentraxins and mannose binding lectin. Similarly, antibody-based therapy, adoptive immunotherapy and vaccination may all prove useful in the future.<sup>146</sup>

## Therapeutic approaches

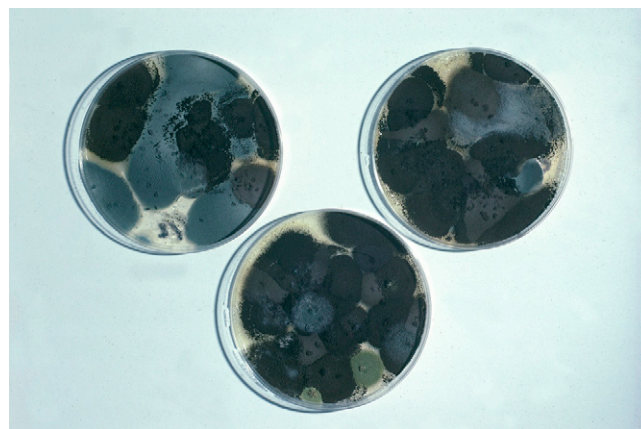
### Prevention

Strategies to minimize the density of airborne conidia within healthcare institutions include: (1) HEPA filtration; (2) laminar airflow within isolation rooms; (3) provision of a well-sealed room; (4) positive room pressure; and (5) frequent air changes within rooms.<sup>79</sup>

There is no definitive evidence to support the use of HEPA filtration for the prevention of invasive aspergillosis.<sup>80</sup> HEPA filters must be regularly monitored and changed to ensure optimal performance. The adequacy of HEPA filtration and the extent of environmental contamination can be investigated using air sampling techniques.

Other issues which may have an impact upon the environmental fungal burden include cleaning practices, hospital construction and renovation, the provision of certain foods (cereals, nuts, and spices may be contaminated with *Aspergillus*; Fig. 11-11), and the use of potted plants and flowers within patients' rooms.<sup>147,149</sup> Each of these factors may need to be addressed by infection control teams and hospital infection control policies.

The construction, renovation, repairs and demolition of buildings produce fungal environmental contamination and may lead to an increased risk of invasive aspergillosis in susceptible patients. A multidisciplinary team which includes infection control staff should be convened to oversee any potential



**Figure 11-11** *Aspergillus fumigatus* and *Aspergillus niger* growing from tea bags of Darjeeling tea.



problems. A program of active surveillance for fungal contamination should be implemented. In addition, a regular review/audit of all histopathologic, microbiologic and mortality data should be undertaken to ensure there are no additional cases of invasive aspergillosis. If at all possible, susceptible patients should not be treated in areas where there is construction or demolition activity. If patients cannot be relocated, an attempt should be made to seal air intakes adjacent to construction, seal windows and minimize the number of open doors and entrances. Dust control measures within corridors also represent a simple but important infection control measure.<sup>147</sup>

## Prophylaxis

The term “prophylaxis” should be reserved for the administration of antifungal agents to patients who are (or will be) at risk of IFI (including invasive aspergillosis), but do not demonstrate any clinical features of current infection. Antifungal prophylaxis is frequently used in HSCT recipients, patients with prolonged neutropenia and for solid organ transplant recipients (especially lung transplantation). While the majority of patients will not develop invasive aspergillosis, the devastating consequences of infection usually outweigh the risk of adverse drug-related events and the added cost of drug administration.

Historically, fluconazole has been used as a prophylactic agent in post-BMT settings, despite the absence of any inherent anti-*Aspergillus* effect. Two randomized clinical trials have compared itraconazole with fluconazole for prophylaxis in allogeneic bone marrow transplantation.<sup>150,151</sup> These studies suggest that itraconazole is at least as efficacious as fluconazole in preventing invasive candidiasis, superior in decreasing the rate of invasive aspergillosis, and may decrease the rate of death associated with invasive aspergillosis. A favorable outcome appears to be dependent on achieving adequate serum levels<sup>127</sup> and in this regard itraconazole suspension provides an advantage over capsules. Importantly, however, itraconazole suspension is less well tolerated than fluconazole, and causes gastrointestinal intolerance and deranged liver function tests. The use of itraconazole may be optimized by: (1) using a dosage of 200 mg bd; (2) commencing prophylaxis after conditioning chemotherapy (to minimize cumulative hepatotoxicity); and (3) switching patients who are intolerant of itraconazole solution to the IV formulation to ensure continued antifungal coverage.<sup>152</sup>

The efficacy of posaconazole as a prophylactic agent in patients with neutropenia in comparison with itraconazole is not known (a recent trial compared posaconazole with fluconazole or itraconazole).<sup>153</sup> Nevertheless, posaconazole is likely to have an increasing role as a prophylactic agent in high-risk patients.<sup>153,154</sup> Micafungin appears a useful alternative to fluconazole as prophylaxis for patients undergoing HSCT; the administration of micafungin results in significantly fewer invasive fungal infections.<sup>137</sup> While there appear to be fewer cases of invasive aspergillosis in patients receiving micafungin, this difference did not reach statistical significance.

Aerosolized amphotericin B has been used as a means of circumventing the side effects of systemic administration of amphotericin B. Nebulized amphotericin B is used widely in lung transplant recipients, but has also been used in HSCT recipients.<sup>155</sup> Nebulized amphotericin B deoxycholate is associated with bronchospasm, poor taste, nausea and vomiting. ABLC

appears to be better tolerated than amphotericin B deoxycholate.<sup>156,157</sup> The intrabronchial pharmacokinetics, optimal dosage and overall utility of this approach require further study.

## Empiric and preemptive therapy

The terms “empiric” and “preemptive therapy” refer to the treatment of high-risk patients who have some clinical, radiologic or laboratory evidence of an IFI.<sup>158</sup> Empiric therapy refers to the administration of an antifungal agent to a patient with prolonged neutropenia and fever unresponsive to broad-spectrum antibacterial agents.<sup>158</sup> This approach represents the standard of care in most institutions, although there is a recognition that fever is a very non-specific sign, and the majority of patients do not have an IFI. Amphotericin B deoxycholate has been the standard comparator following the use of this agent in the original EORTC trials conducted over 20 years ago.<sup>159,192</sup> Comparable efficacy but superior tolerability have been demonstrated for itraconazole and liposomal amphotericin B. Voriconazole did not meet prespecified non-inferiority endpoints and has not been routinely used in this setting.<sup>161</sup> More recently, caspofungin has been demonstrated to be non-inferior to the administration of liposomal amphotericin B 3 mg/kg and thus is an alternative agent for the treatment of this syndrome.<sup>136</sup> These trials have generally demonstrated that newer antifungal agents are better tolerated than amphotericin B deoxycholate, but of equivalent efficacy.

Given the non-specific nature of fever as an indicator of IFI, a more refined approach may be the use of other surrogate markers of infection to guide antifungal therapy. In this regard high-resolution CT scans, PCR and galactomannan in blood have been used as triggers for the initiation of antifungal therapy. These techniques may enable the administration of specific anti-*Aspergillus* agents in the early phases of infection, while safely withholding therapy in uninfected patients. There are important limitations to each of these approaches which means they have not been widely embraced (a thoracic CT will not detect extrapulmonary disease, a standardized PCR assay is not commercially available, and the negative and positive predictive value of galactomannan in certain settings may be too low to confidently guide preemptive antifungal therapy). Nevertheless, there is wide recognition that such an approach is required to further stratify patients in terms of their risk of invasive aspergillosis, and is likely to be increasingly used with improvements in diagnostic modalities.

## Treatment of established invasive aspergillosis

### General principles

The management of acute invasive aspergillosis has undergone significant changes in recent years with the advent of new drugs, formulations and classes. The prompt initiation of antifungal therapy is likely to be critical for optimizing the therapeutic outcome<sup>162, 163</sup> but remains difficult to achieve using current diagnostic modalities. Restoration of host immunity is also a critical facet of management.<sup>146</sup> Surgical intervention may be beneficial in certain instances.

The drugs of choice for the treatment of disseminated aspergillosis are voriconazole and liposomal amphotericin B. Voriconazole should be administered IV 6 mg/kg bd IV on

day 1 and then either 4 mg/kg bd IV or 200 mg bd orally as maintenance therapy.<sup>128</sup> The superiority of this agent over conventional amphotericin B, in terms of both survival and the response to therapy, was demonstrated in a large randomized clinical trial.<sup>128</sup> The optimum duration of treatment has not been established, and is likely to vary widely for individual patients. Certainly, treatment should continue beyond neutrophil recovery, until there is resolution (or clear stabilization) of any clinical and radiologic abnormalities. Continuation of therapy through subsequent episodes of immunosuppression is warranted (secondary prophylaxis). An alternative to voriconazole is liposomal amphotericin B which should be administered at 3 mg/kg/day. In a recent clinical trial conducted in profoundly immunocompromised patients a response rate of 50% was seen and a 12-week survival rate of 72%.<sup>164</sup> While there are no randomized clinical trial data, itraconazole has demonstrated efficacy for the primary and salvage treatment of IA.<sup>165,166</sup> The utility of the echinocandins as first-line therapy for invasive aspergillosis has not been established. When used as salvage therapy, there is approximately a 45% response rate, which is comparable to other antifungal agents when studied in this setting.<sup>138</sup>

Combining antifungal agents is increasingly touted as an approach to improve outcome of patients with invasive aspergillosis. While this strategy is increasingly (and in many cases routinely) used in clinical practice, there are no data from randomized clinical trials to support this practice. Retrospective studies and results from laboratory animal models suggest that the combination of a triazole and echinocandin may be beneficial.<sup>139,167,168</sup> Recent evidence from a well-validated neutropenic rabbit model of invasive pulmonary aspergillosis suggests that the combination of a triazole and polyene may be antagonistic, and should be avoided if possible.<sup>169</sup> The consequences of sequential azole and polyene therapy remain poorly elucidated.

The requirement for other adjunctive therapies such as surgery and immunotherapy should be considered in individual cases, especially for patients who are failing therapy with conventional agents and approaches.

## Treatment of individual aspergillus syndromes

### Allergic bronchopulmonary aspergillosis

Mild disease may not require systemic therapy. For more severe disease prednisolone improves symptoms, leads to a clearing of pulmonary infiltrates, and improves spirometry. A dose of 1 mg/kg should be used until clinical and radiologic resolution is observed, with a rapid tapering to a minimal maintenance dosage. Itraconazole has a role as a steroid-sparing agent and should be considered in all patients with ABPA.<sup>170</sup> Inhaled bronchodilators, inhaled steroids and postural drainage may all be indicated and should be considered.

### Aspergilloma

Successful medical therapy for aspergilloma is limited by the inability of systemically administered agents to penetrate to the infected site. Nevertheless, long-term maintenance therapy with a triazole, such as itraconazole, may be indicated if there are significant symptoms and surgery is not feasible. Surgical

resection should be considered in all patients with aspergilloma, and especially in the setting of significant hemoptysis. If surgical resection is not possible, percutaneous instillation of amphotericin B may temporarily ameliorate symptoms (see [www.aspergillus.org.uk](http://www.aspergillus.org.uk) for formulation of amphotericin B paste). Cases of recurrent or large-volume hemoptysis may also be managed by bronchial artery embolization.

### Chronic necrotizing aspergillosis of the lung

The role of parenteral antifungal agents for this condition is limited given that most patients require life-long maintenance therapy. A short induction course (1–2 weeks) of a polyene, triazole or echinocandin may be reasonable strategy in some patients, although substantial improvement in either respiratory symptoms or radiologic abnormalities within this time-frame is unlikely. Surgery may need to be considered, but is often precluded because of extensive involvement of the lung, poor respiratory reserve and the potential of intraoperative seeding of the pleural space. Long-term therapy with an *Aspergillus*-active triazole, such as itraconazole or voriconazole, represents the mainstay of therapy.<sup>97,171</sup> Careful consideration should be given to the possibility of an unrecognized underlying condition, such as neoplasia or secondary bacterial infection. Atypical mycobacterial infections appear particularly common, and should be actively sought and treated using an antituberculous regimen not containing rifampicin (due to an interaction with triazoles). The intracavitary administration of amphotericin B paste and bronchial artery embolization may be appropriate in individual cases.

### Infection of the paranasal sinuses

A combined medical and surgical approach is frequently indicated. Patients with acute *Aspergillus* sinusitis often require (multiple) surgical debridements and treatment with systemic antifungal therapy. Voriconazole is the drug of choice, but the lipid preparations of amphotericin B are suitable alternatives. The therapeutic approach to chronic invasive sinusitis is similar. Extensive and recurrent debridement may be required in addition to prolonged antifungal therapy. An appropriate approach may be the administration of a parenteral antifungal agent at the time of debridement surgery (using a triazole or polyene), with a much longer period of consolidation therapy with a triazole.

### CNS aspergillosis

The therapeutic outcome of CNS aspergillosis prior to the introduction of new drugs and drug classes was extremely poor, with near 100% mortality.<sup>172</sup> Voriconazole penetrates the CNS and can be found within the CSF in laboratory animals.<sup>173</sup> The response to voriconazole therapy is approximately 35%.<sup>174</sup> Neurosurgical treatment may also be an important facet of management and may include the drainage of abscesses and the insertion of shunts to manage hydrocephalus.<sup>174</sup>

### Ocular aspergillosis

Patients with *Aspergillus* keratitis may be treated with topical natamycin 5%.<sup>175</sup> Amphotericin B may be used, but there are no commercially available ocular preparations; a 0.15–0.3%

solution can be made from IV powder using water as the diluent.<sup>175</sup> Itraconazole 1% cream can be used to treat *Aspergillus* keratitis.<sup>175</sup> There are a number of recent reports suggesting that topically applied voriconazole may also be useful.

The treatment of endophthalmitis is more problematic. These cases should be treated systemically and a search made for other infectious foci, since the majority of cases result from hematogenous dissemination. A vitrectomy may need to be considered with the intravitreal instillation of antifungal agents. The intravitreal instillation of amphotericin B may cause retinal necrosis and detachment.<sup>175</sup> Intravitreal itraconazole has not been used in the clinical context. There are a number of cases in which the intravitreal instillation of voriconazole has been successfully used.<sup>176</sup>

## Endocarditis

The successful treatment of *Aspergillus* endocarditis requires aggressive surgical debridement, including the removal/revision of prosthetic devices, and long-term medical therapy. Voriconazole should probably be considered the first-line agent, although definitive data are absent; lipid formulations of amphotericin B may also be appropriate. The appropriate length of therapy is not known, but a period of long-term consolidation therapy is frequently used.

## Osteomyelitis

Surgical debridement of necrotic tissue is critical for the management of *Aspergillus* osteomyelitis. Prolonged consolidation therapy with itraconazole (400 mg/day) or voriconazole has been demonstrated to be successful.<sup>177,178</sup>

## Cutaneous aspergillosis

Both primary and secondary cutaneous aspergillosis should be treated with systemic antifungal therapy. Surgical debridement may be an important adjunctive therapy.

## Prospects of vaccines for invasive aspergillosis

Vaccine development for invasive aspergillosis is limited by our understanding of effective host responses and by limitations in our knowledge of fungal molecules that elicit protective immunity. Nonetheless, the past few years have witnessed advances in our understanding of both the immune response to *Aspergillus* and the relationship between antigenicity and the ability to confer protection. Manipulations that promote the development of Th1-associated responses correlate with increased resistance to disease, at least partly because of consequent enhancement of innate cellular effector function. Two areas of investigation most actively being pursued include the search for adjuvants that will allow products of *Aspergillus fumigatus* to become effective vaccine candidates, regardless of the form of immunity they ordinarily induce, and the identification of the specific antigens that will most effectively elicit beneficial responses, as reviewed by Feldmesser.<sup>179</sup> Strategies using antigen-exposed dendritic cells as adjuvants appear to be particularly promising. Though we currently are far from identifying a candidate that is applicable for human trials, recent progress is encouraging.

## Antifungal sensitivity and resistance

Although the arsenal of agents with anti-*Aspergillus* activity has expanded over the last decade, mortality due to invasive aspergillosis remains unacceptably high. *A. fumigatus* still accounts for the majority of cases of invasive aspergillosis; however, species of *Aspergillus* other than *A. fumigatus* that are less susceptible to antifungals have begun to emerge. Antifungal drug resistance of *Aspergillus* might partially account for treatment failures.

Recent advances in our understanding of the mechanisms of antifungal drug action in *Aspergillus*, along with the standardization of in vitro susceptibility testing methods, have underlined the importance of resistance testing. In addition, molecular biology has started to shed light on the mechanisms of resistance of *A. fumigatus* to azoles and the echinocandins, while genome-based assays show promise for high-throughput screening for genotypic antifungal resistance.

Several problems remain, however, in the study of this complex area. Large multicenter clinical studies, point prevalence or longitudinal, to capture the incidence and prevalence of antifungal resistance in *A. fumigatus* isolates are lacking. Correlation of in vitro susceptibility with clinical outcome and susceptibility breakpoints has not been established. In addition, the issue of cross-resistance between the newer triazoles is of concern. Furthermore, in vitro resistance testing for polyenes and echinocandins is difficult, and their mechanisms of resistance are largely unknown.

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# Zygomycosis

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## Introduction

The term “zygomycosis” encompasses various types of infections, often categorized under the names “mucormycosis,” which refers to diseases caused by members of the order Mucorales and Entomophthorales (Table 12-1). We have elected to use the term “zygomycosis” for all infections caused by fungi that are classifiable as zygomycetes. Mucoraceous zygomycetes are cosmopolitan phylogenetically related even though they are responsible for a broad spectrum of infections that are somewhat infrequent but often fatal. The principal forms of these diseases include rhinocerebral, pulmonary, cutaneous, and disseminated infections. The main pathophysiologic mechanism is vascular invasion and necrosis, being often associated with a poor prognosis. Populations at risk are mostly immunocompromised subjects, such as patients with neutropenia, immunosuppressive therapy, malignancies, chronic diseases such as diabetes, stem cell and solid organ transplants and chronic renal failure. Zygomycoses caused by members of the Entomophthorales, on the other hand, are infections that are usually diagnosed in patients living in tropical areas. These infections are usually chronic, subcutaneous or intraabdominal and limited, without blood vessel invasion. They have a much better prognosis than infections caused by mucoraceous zygomycetes. In general, there are limited therapeutic options for these infections, often requiring a combination of aggressive surgical debridement and antifungal therapy.

## Infections with fungi of the order mucorales

### Ecology and transmission

Most of the zygomycetes have a wide geographic distribution,<sup>1,2</sup> in which they use a variety of substrates as nutrient sources. All the pathogens are thermotolerant in that they are able to grow at temperatures greater than 37°C. Members of the order Mucorales are found in decaying vegetables, foodstuffs, fruits, soil, and animal excreta. Most of them, especially *Rhizopus* spp., are able to rapidly grow on high-concentration carbohydrate substrates. Sporangiospores are released into the environment as airborne propagules that can contact a range

of surfaces. The major clinical settings are rhinocerebral and pulmonary, owing to the inhalation of sporangiospores with subsequent dissemination from the respiratory tract. Large numbers of airborne propagules can result in contamination.

Nosocomial infections have resulted from sporangiospores and hyphae present as contamination of air-conditioning systems and wound dressings.<sup>3,4</sup> Peritonitis after peritoneal dialysis,<sup>5</sup> disseminated infections after infusion of contaminated fluids, skin infection after intravenous catheter use, and other infections related to foreign bodies like artificial heart valves and contact lenses have also been reported.<sup>2</sup> Nosocomial infections caused by mucoraceous fungi are not as frequent as those caused by *Aspergillus* spp.; many reports involve patients with hematologic malignancies. There is no person-to-person transmission.

### Risk factors

The underlying disease is more important in the development of infection than other host factors such as race, age or gender. Although the infection rate in male and female is generally equal,<sup>2,6,7</sup> a recent metaanalysis showed a slight predominance of infection in males (65%).<sup>102</sup> The median age of patients with these infections is 40 years<sup>102</sup> and although infections caused by mucoraceous fungi occur in newborns to old age, there is a relative higher proportion of children among patients without underlying conditions than in any other group.<sup>2</sup>

Predisposing factors have been classically described and are listed in Table 12-2. Recent years have seen an exponential growth of the deeply immunocompromised populations, such as the stem cell and solid organ transplant recipients, as the populations that are most frequently affected by these infections.<sup>103</sup> Other risk factors include: diabetes, metabolic acidosis or hyperglycemia, corticosteroid therapy, immunosuppressive therapy for organ or bone marrow transplantation, neutropenia, trauma, HIV, and deferoxamine therapy for iron or aluminum overload.<sup>2,6-13</sup> Perhaps one of the most important risk factors is diabetes mellitus, especially when ketoacidosis is present. The increased concentration of glucose stimulates rapid zygomycete growth. The growth rate is so fast it seems that these fungi do not have either time or a need to form septa in their hyphae.



**Table 12-1** Common zygomycetes in clinical practice

Mucorales	Entomophthorales
Mucoraceae	Entomophthoraceae
<i>Absidia</i> spp.	<i>Conidiobolus</i> spp.
<i>Mucor</i> spp.	Basidiobolaceae
<i>Rhizomucor</i> spp.	<i>Basidiobolus</i> spp.
<i>Rhizopus</i> spp.	
Cunninghamellaceae	
<i>Cunninghamella</i> spp.	
<i>Saksenaia</i> spp.	

**Table 12-2** Risk factors for zygomycosis

Immunocompetent patients	Immunocompromised patients
<ul style="list-style-type: none"> <li>Major trauma and contaminated lacerations</li> <li>Surgery and use of contaminated materials</li> <li>ICU stay with high acuity</li> <li>Intravenous drug use</li> <li>Deferoxamine therapy</li> </ul>	<ul style="list-style-type: none"> <li>Diabetes mellitus/ketoacidosis</li> <li>Hematologic malignancies (leukemia, lymphoma, myelodysplastic syndrome)</li> <li>Stem cell transplantation</li> <li>Solid organ transplantation</li> <li>Chronic renal failure</li> <li>Immunosuppressive agents</li> </ul>

The influence of deferoxamine therapy in the pathogenesis of infections caused by mucoraceous fungi has been known since the late 1980s.<sup>14-16</sup> Diabetes was a predisposing factor in 36% of the 255 cases of mucoraceous infections compiled by R. D. Baker.<sup>6</sup> In more recent reviews, diabetes was found in 40–46% of cases, leukemia and lymphoma in 15–21%, solid tumors or renal failure in 5%, miscellaneous conditions in 20%, and 9% of patients had no documented underlying illness.<sup>7,13</sup> Experimental animal data have been used to show that deferoxamine therapy, diabetes, and neutropenia are clearly predisposing factors to infections caused by members of the Mucorales. Pretreatment with deferoxamine shortened the survival of animals experimentally infected with *Rhizopus arrhizus* and *R. microsporus* var. *rhizopodiformis*.<sup>24</sup> Zygomycetes have varying degrees of in vitro sensitivity to deferoxamine. The growth of *R. microsporus* and *Cunninghamella bertholletiae* was stimulated by deferoxamine, whereas the growth of *R. arrhizus*, *Absidia corymbifera* and *Mucor circinelloides*<sup>25</sup> was hindered.

Corticosteroid treatment does not favor infection by *R. arrhizus*.<sup>26</sup> Bronchoalveolar macrophages from normal mice are able to handle germinating sporangiospores of *R. arrhizus* whereas those in diabetic mice are not. Human neutrophils and their products are able to kill hyphae of *R. arrhizus*, which partly explains the susceptibility of neutropenic patients to zygomycetes.<sup>27</sup>

A recently recognized risk factor is human immunodeficiency virus (HIV) infection. In a review of 28 cases of zygomycosis in this setting,<sup>13</sup> 16 of 22 patients were intravenous drug abusers, a risk factor recognized in HIV-negative individuals.<sup>6,17,18</sup>

Although controversial, one of the most recently described risk factors is prophylaxis or therapy with the antifungal voriconazole. This agent does not have activity against the zygomycetes, and there are a few reports in which its use has been associated with breakthrough fungal infection with zygomycetes. However, at this time it is unclear if this relationship is indeed causal or if this is a reflection of the increasing incidence of this infection in those immunocompromised populations.<sup>104</sup>

Small outbreaks of *Rhizopus microsporus* var. *rhizopodiformis* infections have been linked to contaminated Elastoplast bandages<sup>3,4,19</sup> an adhesive pad covering a jejunostomy,<sup>20</sup> and airborne propagules.<sup>21</sup> In a review of cutaneous mucoraceous infections, 15 cases were associated with the use of needles, including vascular or tissue infusion catheterization sites (eight cases), insulin injection sites (three cases), and biopsy sites (three cases).<sup>22</sup> Recently, wooden tongue depressors used to construct splints for intravenous and arterial cannulation sites were identified as the source of infection caused by *Rhizopus microsporus* in preterm infants in a nursery unit.<sup>23</sup>

Finally, immunocompetent hosts can also be (very rarely) infected.<sup>7,13</sup> Local injury has been documented before the presence of infection that ranged from cutaneous to rhinocerebral and disseminated infections.

## Virulence factors

Zygomycoses are rare in healthy individuals, unless trauma has provided a portal of entry for the fungus. This suggests that fungal virulence factors are operative when some of the host defense mechanisms are altered.<sup>28</sup>

## Underlying disease, portal of entry, and clinical features

Ketoacidosis seems to predispose individuals to sinus infections. Malignancies, profound neutropenia, and corticosteroid therapy are often associated with pulmonary and disseminated infections. Half of the 59 cases associated with deferoxamine therapy in the report by Boelaert and colleagues were disseminated infections, the second most common presentation being the rhinocerebral form.<sup>16</sup>

Hematologic malignancies predominate among the risk factors for disseminated infection. Breakdown of physical barriers such as skin, the gastrointestinal tract, and lungs has been incriminated in intravenous drug abusers and neonates. Disseminated infections from gastrointestinal localization occur in children with malnutrition, malabsorption or diarrhea and in adults with peptic ulcers, bowel abnormality after surgery, trauma, inflammatory disease or underlying liver disease.<sup>29</sup> Gastrointestinal infections are sometimes associated with the ingestion of contaminated food.

In solid organ transplant recipients, zygomycetes have been reported to cause infection in 1–9% of patients<sup>30</sup> at a median time of 60 days (range, 2 days to 4 years) after transplantation.<sup>31</sup> Corticosteroid use and diabetes mellitus were associated with 10 and 5, respectively, of the 14 cases reviewed in 1986.<sup>31</sup> In transplant recipients, all clinical forms of this disease can be seen.

In acquired immunodeficiency syndrome (AIDS), the most frequent clinical presentations include renal (seven cases), cutaneous, and sinoorbital and cerebral (five cases each)

infections.<sup>13,32</sup> Intravenous drug abuse is clearly predisposing to cerebral infection.<sup>17</sup> Among 16 HIV-infected intravenous drug addicts, four had isolated cerebral infections, a figure that is less than that recorded (16 of 19) for HIV-negative IV drug abusers.<sup>13</sup> Typically, involvement of the basal ganglia is seen during cerebral mucoraceous infections in this latter population.<sup>17</sup>

In a review of 111 cases of cutaneous zygomycoses, local factors included surgery (17%), burns (16%), motor vehicle-related trauma (12%), use of needles (13%), knife wounds (3%), insect or spider bites (3%), and other kinds of trauma or skin lesions (23%).<sup>22</sup> Despite the fact that the sites were initially the skin and the patients were healthy, dissemination of the etiologic agents and a fatal outcome still occurred in some patients. This was especially true if the diagnosis was delayed by inappropriate diagnostic procedures or belief that the cultured fungus was a contaminant.<sup>33</sup> Infections of the skin are also seen in patients with preexisting illnesses.

## Etiologic agents

One of the major problems concerning zygomycosis is the identification of the organism involved. When reviewing the literature, and especially the older reports, information regarding the etiologic agent is often missing; only histopathologic findings support the diagnosis of zygomycosis. In a review of 361 cases reported between 1958 and 1985, Espinel-Ingroff and collaborators found only 156 cases in which the identification (129 Mucorales and 27 Entomophthorales) was reported.<sup>7</sup> Among them, *Rhizopus* spp. were the most frequent organisms (95 cases) followed by *Mucor* spp. (19 cases). Even when the identifications are provided, the details necessary to ensure the identification is correct are frequently not provided. Species that were once considered contaminants, such as *Apophysomyces elegans*, are now being reported as the etiologic agents of infection. Although some species are more commonly associated with particular clinical settings (*R. arrhizus* and rhinocerebral infection), most of these fungi can cause a wide variety of infections. Hence, precise identification is needed.

Among the Mucorales, members of the family mucoraceae are most frequently involved in human diseases. *R. arrhizus* (previously referred to as *R. oryzae*) is the most frequent agent of rhinocerebral forms. *R. microsporus* var. *rhizopodiformis* accounts for 10–15% of human infections, primarily causing cutaneous and gastrointestinal infections.<sup>1</sup> This species is commonly associated with nosocomial zygomycosis.<sup>4,20,23,34</sup> Despite the fact that it is often cited as a common agent of mucoraceous infection in humans, *A. corymbifera* is rarely reported in the literature.<sup>35</sup> Strangely, it was the organism identified in five of 17 infections in patients with AIDS for which the etiologic agent was identified (three focal kidney infections, one pharyngeal ulceration, and one cutaneous infection).<sup>13,32</sup>

Until 1993, *Rhizomucor pusillus* was incriminated in 12 cases of fatal zygomycosis, including rhinofacial (three cases), disseminated (six cases), and pulmonary (three cases) infections.<sup>21</sup> *Rhizopus microsporus* var. *microsporus* has rarely been isolated from human lesions<sup>36</sup> as has *Rhizopus azygosporus*.<sup>37</sup> *Mucor* spp. are rare causes of disseminated disease,<sup>38</sup> but they have been recovered from cutaneous lesions,<sup>39,40</sup> endocarditis,<sup>41</sup> and arthritis.<sup>42</sup>

Until 1985, only seven cases of *Cunninghamella bertholletiae* infections were reported.<sup>43</sup> Since then, several reports have underlined the importance of this fungus as an agent of pulmonary and disseminated infections in immunosuppressed patients. These infections have a severe prognosis, with only three of 21 patients surviving.<sup>8,9,44,45</sup> Two cases of cutaneous oarticular infection recorded in AIDS patients were caused by *C. bertholletiae*.<sup>13</sup>

*Apophysomyces elegans* and *Saksenaea vasiformis* have been recovered from skin and bone lesions after traumatic mechanical injuries<sup>46–51</sup> or burns.<sup>52</sup> Both have also been associated with other forms of infections in healthy patients, including cases of acute invasive rhinocerebral zygomycosis caused by *A. elegans*<sup>53</sup> and *S. vasiformis*<sup>54</sup> and disseminated infection caused by *S. vasiformis*.<sup>55</sup> Both of these fungi often cause infections in patients living in warmer climates.<sup>10,56</sup> *Apophysomyces elegans* grows well in warm soil. Several cases involved highway accidents. Interestingly, they are mostly associated with infections in previously healthy hosts.

*Cokeromyces recurvatus* has been reported to colonize mucosal surfaces,<sup>57</sup> cause chronic cystitis,<sup>58</sup> and has possibly contributed to a fatal outcome in a patient having diabetes and perforated peptic ulcer.<sup>59</sup> This is a rare opportunistic pathogen.

## Infections with fungi of the order entomophthorales

### Epidemiology and transmission of *Basidiobolus ranarum*

Infections due to *Basidiobolus ranarum* are reported mainly in tropical areas of Asia (India, Indonesia, and Myanmar), Africa (Uganda, Nigeria, Cameroon, Togo, Ivory Coast, Sudan, Senegal, Somalia, and Kenya), South America (mostly Brazil), North America (Mexico),<sup>60</sup> and recently Australia.<sup>61</sup> One well-documented case reported in England occurred in a patient who contracted the organism in Indonesia.<sup>60</sup>

The fungus occurs in decaying vegetation, soil, and as a saprobe in the intestinal contents of various insectivorous reptiles (lizards, chameleon), amphibians (toads), and mammals (bats, kangaroos, and wallabies).<sup>62,63</sup> The portal of entry is believed to be the skin after insect bites, scratches, and minor cuts. This helps to explain the most common presentation in young children involving the thighs and buttocks. However, there is rarely a history of previous trauma. Patients usually have no evident underlying disease, although rare cases have been described in immunocompromised patients, which mimic infections caused by the mucoraceous fungi.<sup>64,65</sup> A case after intramuscular injection has been reported.<sup>66</sup>

Infections caused by *Basidiobolus ranarum* are mainly diagnosed in children (80% under the age of 20 years)<sup>60</sup> with a male/female ratio of 3:1.

### Epidemiology and transmission of *Conidiobolus coronatus*

*Conidiobolus coronatus* infections have been reported from tropical portions of Africa (mostly Cameroon and Nigeria, but also Chad, Zaire, Kenya, Central African Republic, Guinea)

and the Americas (Costa Rica, Caribbean islands, Columbia, Brazil).<sup>67</sup>

The fungus is found in decaying wood, plant detritus, on insects, and in the gastrointestinal tract of lizards and toads. There are seasonal variations in the yield of *C. coronatus* from soil, a maximum being recorded in September and October, which suggests an influence of climate on spore survival, which may also help explain the geographic distribution of the infection.<sup>60</sup> The spores are believed to enter the body by inhalation and then invade tissues through wounded nasal mucosa.

There is a male/female ratio of 10:1 and a predominance of the disease among young adults. Infection is rare among children.<sup>67</sup> There is no known underlying predisposing factor for the infection.

### Epidemiology and transmission of *Conidiobolus incongruus*

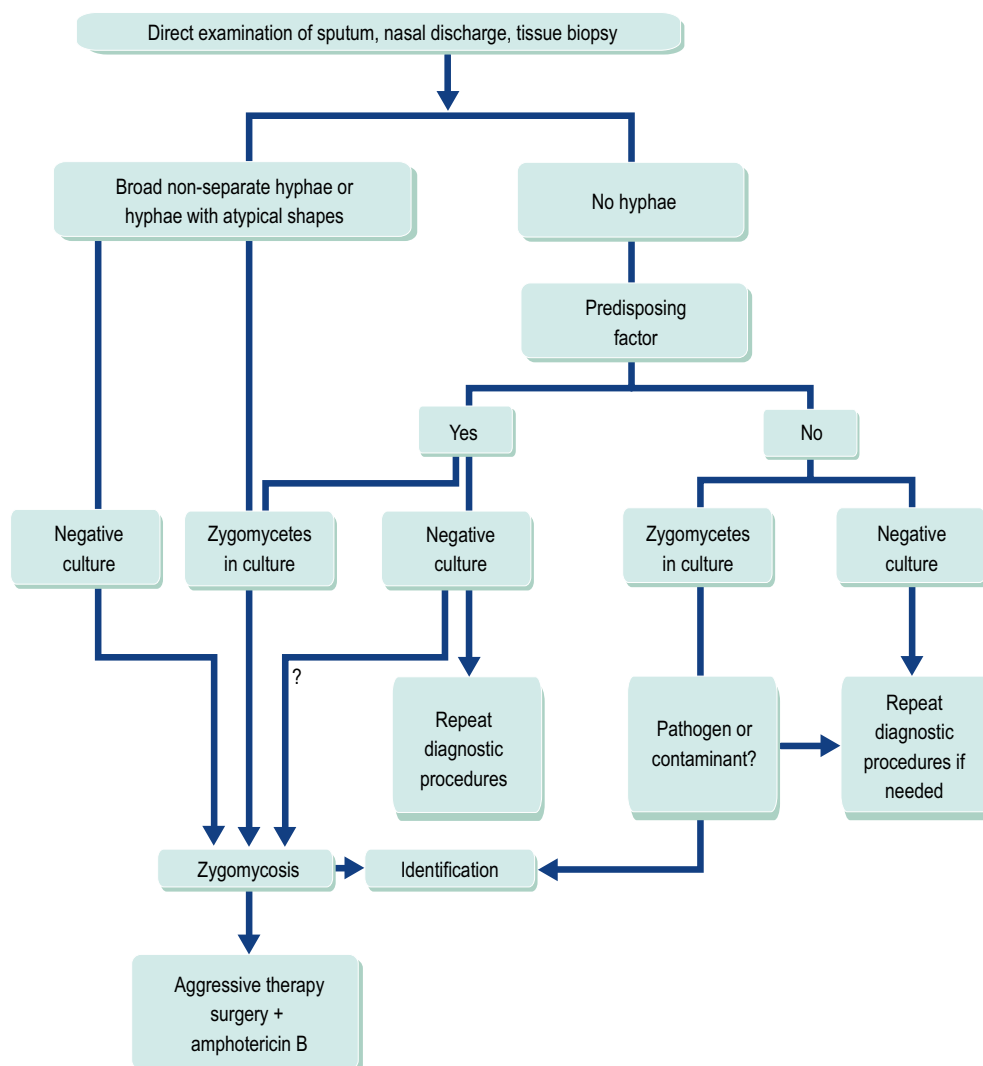
The infection is rare, with only a few cases reported from the United States.<sup>65</sup>

### Laboratory diagnosis

Because of the dreadful prognosis of zygomycosis, the smallest suspicion of the disease in a patient at risk should prompt biopsy to obtain tissue samples that will allow for direct microscopic examination, histologic study, and culture. Because the fungi responsible for these infections can be contaminants in laboratories, isolated cultures without demonstration of the broad hyphae in tissues or samples are difficult to interpret unless the patient is neutropenic or diabetic. A positive culture of a zygomycete from sputum, skin scraping or nasal discharge is more meaningful in the presence of predisposing factors or direct microscopic examination of the material (see Fig. 12-1).

### Direct examination

Samples should be obtained from sites that look infected. In the rhinocerebral form, scrapings of nasal mucosa and aspirates of the sinuses should be obtained. For infections involving the lungs, sputum or centrifuged bronchoalveolar lavage fluid is



**Figure 12-1** Diagnostic steps for zygomycosis.



useful. From any site, biopsy of necrotic infected tissues should be obtained. Specimens should be observed with a microscope after mounting the material in a few drops of potassium hydroxide (KOH) and gently heating the slide to clear the tissue.<sup>2</sup> Broad (7–15 µm), sparsely septate hyphae can be seen. Swollen cells (up to 50 µm) and distorted hyphae are often seen. Branching differs from that of *Aspergillus* spp. by the fact that it occurs frequently at a 90° angle to the main hyphae. The diagnosis cannot be ruled out by other appearance (thinner hyphae, less than 5 µm, or sharper branching) or by absence of hyphae, because the fungal elements are often scattered in tissues. The unusual appearance of zygomycetes also includes yeast forms of *Mucor circinelloides* that can be mistaken for *Paracoccidioides brasiliensis* in fluid specimens.<sup>68</sup>

## Histology

During acute infections caused by mucoraceous fungi, tissue is necrotic, hemorrhagic or pale because of invasion of blood vessels by the fungus that leads to thrombosis, necrosis, and infarction. Inflammation is absent in most cases. Staining of the fungus can be achieved by hematoxylin and eosin or periodic acid-Schiff. Silver staining using the GMS stain is inconstant. Broad, irregularly branched, twisted hyphae can be observed. Some narrow hyphae may be seen, but they lack dichotomous branching typically associated with *Aspergillus*. Septation is rare compared with what occurs in *Aspergillus*. Cross-sections of large hyphae in tissue can superficially resemble yeast cells similar to that seen with *Aspergillus* infection. Fungal elements usually invade the blood vessels and the surrounding tissue.

In chronic infections caused by mucoraceous fungi, and in almost all cases of infections due to members of the Entomophthorales, a chronic inflammatory process can be seen with small abscesses surrounded by a granulomatous tissue reaction. A strong eosinophilic perihyphal reaction is often observed (Splendore-Hoeppli phenomenon) that is variable in size (2–6 µm).<sup>1</sup> Broad irregular hyphae (4–30 µm) with thin walls and rare septation can be seen, singly or in clusters. There is no invasion of blood vessels or infarction of tissue, as in acute infections caused by the members of the Mucorales.

## Culture

All fungi that cause zygomycosis should be grown on standard laboratory media without cycloheximide. Antibiotics can be used in the isolation medium, especially for highly contaminated materials such as nasal discharge and sputum. Tissue, rather than exudate from the surface of a lesion, should be submitted for culture to differentiate colonization from infection and to increase the culture yield.<sup>33,49</sup> Fluids should be spread on agar plates, and tissue biopsy should be minced and not homogenized. Homogenization in a tissue grinder should be avoided, because it decreases culture yield by destroying hyphae. Some authors have reported that a piece of sterile bread without preservatives placed on the surface of the agar plate, on which the specimen is inoculated, can enhance the recovery of zygomycetes.<sup>1,69</sup> Negative cultures can occur as often as 40% of the time. Repeated sampling is useful in cases of negative culture with positive histologic examination. The growth is rapid and usually visible after 24 hours of incubation at 25–37°C. Once again, the diagnosis of zygomycosis

cannot be established or rejected on culture alone. It depends on a panel of evidence gathered by both the clinician and the microbiologist. After isolation, identification of the fungus often requires the help of a mycologist. Transfer of the sterile isolates to saline agar<sup>46</sup> or to plates containing water supplemented with 1% of filter-sterilized yeast extract solution<sup>70</sup> may help to obtain the characteristic reproductive structures needed for identification. Production of zygospores is sometimes the only means to correctly identify some of these organisms.<sup>71</sup> To overcome the loss of sporulation in *Basidiobolus* species, use of media containing glucosamine hypochloride and casein hydrolysate has been proposed.<sup>72</sup>

## Other means

There is no serologic procedure reliable for the diagnosis of zygomycosis. In 1989, Kaufman and collaborators reported a good specificity (94%) and sensitivity (81%) in an enzyme-linked immunosorbent assay (ELISA) procedure using homogenates of *R. arrhizus* and *R. pusillus*.<sup>73</sup> However, cross-reaction was seen with sera from patients with aspergillosis and candidiasis, a phenomenon that limits the usefulness of the test as a diagnostic tool. Because spores of zygomycetes are present in the environment, detection of antibody reactive with home-made antigens prepared from these fungi will be difficult to interpret, especially in patients at risk for other invasive fungal infections such as neutropenic patients. Given the rapid evolution and often fatal outcome of acute zygomycosis, development of DNA-based diagnostic methods, antigen detection or specific serologic procedures could improve the prognosis of these infections.

## Disease spectrum

Zygomycosis presents a wide spectrum of clinical disease types, depending on underlying conditions of the host and the portal of entry of the fungus. The disease can be acute, one of the most fulminant fungal infections known. In its disseminated or rhinocerebral forms in neutropenic, immunosuppressed or diabetic patients, it can be chronic over years.

## Zygomycosis

In their review of 361 cases of zygomycosis, Espinel-Ingroff and collaborators identified 49% acute and chronic rhinocerebral diseases, 16% cutaneous infections, 11% gastrointestinal infections, 11% pulmonary zygomycosis, 6% disseminated disease, and 9% miscellaneous infections.<sup>7</sup> A more recent metaanalysis by Roden et al,<sup>102</sup> in which 929 cases reported since 1885 were analyzed, sinus infection was the most common presentation (39% of cases), followed by pulmonary infection (24%), and cutaneous infection (19%). Dissemination was reported in 23% of cases in that series.

### Acute rhinocerebral zygomycosis

There is no known sexual or racial predilection; the infection is diagnosed worldwide. The infection begins in the nasal mucosa and extends to the palate, paranasal sinuses, orbit, face, and brain. The usual presenting symptoms of rhinocerebral zygomycosis are acute sinusitis mimicking bacterial sinusitis with

fever and headache often located in the frontal or retroorbital regions. Thick bloody or purulent, usually unilateral, discharge from the nose is present in half of the cases. At this stage, a direct examination of the nasal discharge will often reveal broad irregular hyphae, which confirms the diagnosis. Culture of the specimen, if successful, will often yield *R. arrhizus*.

The subsequent involvement of the contiguous tissues tends to be on the same side as the nasal involvement. Facial pain and edema follow in the next few days with ulceration of the skin surrounding the nose. Black necrotic ulceration of the hard palate respecting the midline can be observed. X-ray examination shows involvement of the maxillary and ethmoid sinuses with a cloudy appearance and air-fluid level.

Spread of the infection to the eye is common and carries a poor prognosis. Orbital pain, diplopia, ophthalmoplegia, proptosis of the eye, lid edema, conjunctivitis, and ulceration of the cornea are observed. Funduscopic examination may reveal normal findings, dilation of the retinal veins, occlusion of the retinal artery, and even hyphae throughout the vitreous.

The fungus has a predilection for invading blood vessels and nerves rather than muscles. This leads to infarction of the invaded areas and extension into the brain. Extension from the sinuses into the brain follows crossing of the dura and, depending on the location and the sequences of events (invasion, thrombosis, infarction of brain tissue), loss of function of cranial nerves, especially the third, fifth and seventh, obtundation or brutal loss of cerebral function can be seen. Thrombosis of the internal carotid artery can also be observed with contralateral hemiplegia. Computed tomographic (CT) scans or magnetic resonance imaging (MRI) are helpful in delineating the extent of the damage and the infection and may guide surgical resection when possible. Apart from the abnormal sinuses, signs of osteomyelitis or bone destruction, mass lesions, signs of infarction, and occlusions can be seen. Examination and culture of the cerebrospinal fluid (CSF) are usually non-contributory.

Lethargy, seizures, and coma are usual complications of brain involvement. Death is common and rapid over the first 1–10 days in refractory or untreated cases. Of the 108 cases of rhinocerebral infections reviewed by Baker in 1971,<sup>6</sup> 44 of 44 patients for whom the duration of symptoms was known to be less than 2 weeks (1–14 days) died from infection compared with 11 of 30 for whom the duration of onset was more prolonged (3 weeks to 10 years). Those who did better usually had no major vascular invasion or brain involvement.

Knowing the sequence of events, observation of an asymmetric facial edema, or the complaint of sudden blurred vision, diplopia, from a diabetic or neutropenic patient, a patient on deferoxamine therapy or an organ transplant recipient should prompt careful examination for early signs of rhinocerebral zygomycosis.<sup>69</sup>

### Pulmonary zygomycosis

In granulocytopenic patients, the infection can be misdiagnosed as invasive aspergillosis. The patients have a fever of unknown origin and pulmonary infiltrates that are refractory to broad-spectrum antibiotics. Chest x-ray is non-specific, showing rapidly progressive bronchopneumonia, segmental or lobar consolidation, signs of cavitation evoking *Aspergillus* infection with air crescent appearance, and rarely pleural effusion. Fungus ball formation resembling aspergilloma can occur. Because of vascular invasion and thrombosis, hemoptysis that is potentially fatal, especially in thrombocytopenic patients,

may develop. Invasion of the contiguous tissues, diaphragm, heart, and mediastinum usually can be found at autopsy. Fistulas (bronchoarterial, bronchopleural or bronchocutaneous) can also complicate the infection.

Diagnosis of pulmonary zygomycosis requires a high degree of suspicion and aggressive management in view of the poor prognosis.<sup>69</sup> CT can determine the extent of the infection and guide stereotaxic biopsies or needle aspirations. Bronchoalveolar lavage and, depending on the patient and the platelet count, brushing or transbronchial biopsies, open lung biopsy by thoracostomy or thoracotomy should be performed. Unless adequate treatment is promptly started, including antifungal therapy, surgical resection when possible, and restoration of immune functions, the evolution of the infection can be rapidly fatal. Among the 49 cases of pulmonary zygomycosis reviewed by Baker,<sup>6</sup> only three survived, and most of the patients died within the first month after the onset. Pulmonary zygomycosis can occur as a component of disseminated or rhinocerebral infection. Its prognosis is even worse.

### Disseminated infections

The clinical manifestations of disseminated zygomycosis are varied, reflecting vascular invasion and tissue infarction in various organs. The disease is rare but occurs in patients immunosuppressed by age, drug therapy or underlying disease, although 11 of 185 cases reported by Ingram and colleagues had no known risk factors.<sup>29</sup> Presenting symptoms are non-specific but point to neurologic, pulmonary or gastrointestinal involvement. Among 113 cases analyzed, 61% had fever, 45% rales or rhonchi, and less than 20% had hepatosplenomegaly, coma or confusion, other neurologic symptoms (palsy or paresis), skin lesions or abdominal tenderness. The diagnosis was rarely suspected before death. Accurate diagnosis depends on histologic examination and culture of the infected tissues. The etiologic agent is rarely reported accurately. Members of the families Mucoraceae and Cunninghamellaceae are predominant. Mortality of disseminated zygomycoses was 96% in the series by Roden et al.<sup>102</sup>

### Cutaneous zygomycosis

Infection of the skin can be a sign of disseminated infection, providing another means of diagnosis through culture and histologic examination of the tissue. Lesions tend to be nodular with an ecchymotic center and a pale surrounding area. The margin of necrosis is often sharp. Ulceration is usually absent.

Cutaneous zygomycosis can also be a localized process that follows traumatic injury, contaminated surgical dressings or colonization of extended and severe burn eschars. The evolution can be chronic, as in the case of an inguinal abscess adjacent to a 2-year-old renal transplant incision.<sup>74</sup> Lesions can be indolent and resolve almost spontaneously or extend into the subcutaneous tissue and become rapidly progressive.<sup>33</sup> Surrounding induration and discoloration are common. Occasionally, the mould can be seen growing on the edge of the wound. Diagnosis requires culture and histopathologic examination of tissue sections to demonstrate invasion of viable tissue. Direct examination and culture of superficial scrapings or swabs are often negative. A rare presentation is pyoderma gangrenosum.<sup>75</sup>

In a review of 111 cases, sites of cutaneous infection included head/neck (14%), thorax (14%), back (9%), one or both upper extremities (24%), and one or both lower extremities (31%). The higher mortality rate (32% vs 15%) was

associated with more centrally located infections, presumably because of the proximity to vital structures and the difficulty of effective debridement.<sup>22</sup> The fact that half of the patients with cutaneous zygomycosis do not have known underlying disease does not improve the prognosis. The death rate was higher (31%) than for patients with diabetes (14%) or other predisposing illnesses (17%). The prognosis of cutaneous infections is still far better than that of other forms of zygomycosis. In a review of 116 cases of zygomycosis, the associated mortality rate was 16% for cutaneous infections vs 67% for rhinocerebral infections, 83% for pulmonary forms, and 100% for disseminated or gastrointestinal infections.<sup>22</sup>

### Gastrointestinal infections

In their review of 185 cases of disseminated zygomycosis, Ingram and collaborators described 16 cases with gastrointestinal disease for whom the disseminated infection started in the gastrointestinal tract, spreading from the bowel or a liver infection.<sup>29</sup> One case of peritonitis associated with invasion of the ileal wall in a patient undergoing continuous ambulatory peritoneal dialysis was more recently reported.<sup>5</sup> The symptoms vary and depend on the extent and localization of the infection. Non-specific abdominal complaints, diarrhea, bloody stools, and hematemesis can be recorded. Involvement of adjacent organs is possible, and outcome depends on the extent of the vascular damage. Gastric infection is easily detectable by gastroscopy, with biopsy of the ulcerative lesions showing broad hyphae.<sup>76,77</sup> Death is common, usually due to massive hemorrhage or perforation.

### Other infections

Any tissue can be infected either contiguously or through hematogenous dissemination. Special note can be made for renal infections in otherwise healthy hosts<sup>78</sup> or in AIDS patients,<sup>32,79-81</sup> osteomyelitis,<sup>56-82</sup> cutaneoarticular,<sup>83</sup> and cardiac infections.<sup>41,84</sup>

Renal infections in otherwise healthy individuals are a rare entity. Two cases have been described,<sup>78</sup> one caused by *Apo-physomyces elegans* that was fatal despite nephrectomy and amphotericin B treatment. Osteomyelitis is described mostly in association with a contiguous tissue infection<sup>35,85</sup> but can also result from hematogenous inoculation<sup>82</sup> or direct contamination after a crushing injury.<sup>79</sup> Arthritis caused by *Cunninghamella bertholletiae* has been described in a patient with AIDS after contusion of the thigh.<sup>83</sup> The patient died 15 days after hospitalization of massive hemorrhage caused by perforation of the femoral artery despite abscess debridement and amphotericin B treatment. Infections of the central nervous system are not limited to direct extension from the nose or the sinuses. In IV drug users, lesions of the basal ganglia are believed to follow intravenous inoculation of the fungus.<sup>17,86</sup>

### Prognosis

The mortality of this infection is extremely high. In the series by Roden et al,<sup>102</sup> it was reported as high as 96% for disseminated diseases, 85% for gastrointestinal forms, 76% for pulmonary infection. Survival was 3% for untreated cases, while 61% of cases treated with amphotericin B alone and 57% of cases treated with surgery alone survived. In a multivariate analysis, the factors associated with worse prognosis were infection with *Cunninghamella* species and disseminated disease.

## Infections with entomophthorales

Three species have been recorded from human diseases: *Basidiobolus ranarum*, *Conidiobolus coronatus*, and *Conidiobolus incongruus*. The infections are also known as basidiobolomycosis and conidiobolomycosis. In contrast to mucoraceous fungi, clinical entities are chronic, often indolent, and not life-threatening infections except in anecdotal cases of disseminated infections. Histologic features are identical, but clinical features differ.

### Infections caused by *basidiobolus ranarum*

The presenting feature is a single painless, unilateral, well-circumscribed subcutaneous mass that usually affects the buttock or the thigh but can also be seen in the arm, the neck, the face or the trunk. The disease starts as a single nodule that progressively grows. The swelling is often described as woody and hard. Extensive lesions can be painful, especially when involving the perineal or perirectal area. Skin color and appearance are normal or erythematous. There is no ulceration and the mass is not adherent to deeper tissues, although involvement of muscle had been described.<sup>66</sup> Enlargement of local lymph nodes is sometimes seen, with the fungus sometimes being cultured from the corresponding biopsy specimens.<sup>87</sup> The lack of draining sinuses, the absence of adherence to underlying structures, and the lack of extension to bone make the differential diagnosis with mycetoma easy. Unusual localization includes gastrointestinal infection.<sup>88</sup>

### Infections caused by *conidiobolus coronatus*

The infection starts in the nasal mucosa and progressively extends to adjacent areas bilaterally, including the nose, cheeks, upper lip, paranasal tissues, and pharynx. The edema affecting all the infected areas leads to significant distortion of the face. Apart from obvious changes in appearance, the patient may complain of nasal obstruction, rhinorrhea, and epistaxis. Invasion of the pharynx may cause dysphagia. The lesion does not usually involve the bones, but maxillary ethmoid sinus obstruction can favor bacterial sinusitis and pain. Invasion of local lymph nodes has been described.<sup>89,90</sup> The evolution of the infection is slow over years. There is no tendency for the mass to ulcerate or become verrucous. However, ulceration of the soft palate has been described and required surgery. The mass is usually anchored to the dermis. There is usually no fever and no biologic signs of infection. Blood cell count and chemistry are normal. Diagnosis is made by culture and histologic examination of biopsied tissues.

Dissemination is rare. In one instance, dissemination occurred in a 64-year-old renal transplant recipient, who died with lesions in the lungs, heart, brain, and kidney.<sup>64</sup>

### Infections caused by *conidiobolus incongruus*

Three cases of infections due to *C. incongruus* have been described so far.<sup>60</sup> One occurred in an immunocompromised patient, in whom the initial pulmonary infection was rapidly fatal after spreading to the pericardium and heart.<sup>65</sup> The two other cases occurred in a 15-month-old boy<sup>91</sup> and a 20-year-old woman<sup>92</sup> with no underlying disease. The infection initially involved the lungs with dissemination to adjacent tissues and eventually caused death of the young woman from massive hemoptysis. The young boy survived after surgical resection



and amphotericin B therapy for 2 months. If the organism had not been cultured, the diagnosis would have easily been that of zygomycosis due to a mucoraceous fungus, although some distinctive histologic tissue reaction can be seen, especially the eosinophilic perihyphal material or Splendore–Hoepli reaction.

## Treatment and prevention

As previously discussed, treatment of acute zygomycosis should not be delayed. The poor prognosis associated with these infections justifies aggressive therapy combining radical surgery, antifungal treatment, and control of the predisposing factors, especially acidosis. Treatment of chronic infections is usually based on antifungal therapy alone. Surgery, when needed, is cosmetic and not indicated until the infection has resolved. The review by Roden et al<sup>102</sup> mentions that survival with any antifungal therapy ranges from 61% to 67%, with surgery alone it is 57%, and the combination of surgery with antifungal treatment has a survival of 70%. Mortality with current antifungals and surgical techniques has decreased from nearly 100% in the 1940s to ~40% on average in the most recent series.<sup>102</sup>

### Treatment of acute zygomycosis

The best management consists of aggressive surgical treatment combined with amphotericin B (or its lipid compounds) and control of the predisposing factors when possible. An 85% survival rate has been reported when patients are treated early with a combination of repeated surgery and aggressive amphotericin B treatment.<sup>7</sup> The key factor to a better prognosis of these infections is an early diagnosis and aggressive therapy, which requires excellent collaboration between the clinician, the surgeon, the pathologist, and the mycologist. The series by Roden et al<sup>102</sup> found that 70% of cases treated with the combination of surgery and antifungals survived the infection.

#### Surgery

CT scan or MRI may help determine the extent of the resection and monitor the efficacy of the treatment. Surgery can include resection of infarcted tissues, extensive debridement or appropriate drainage of sinuses. Debridement may have to be repeated daily for several days.<sup>93</sup> Fatal cases often occur when sites of disease are inaccessible to surgical debridement.<sup>32</sup> In rare cases, localized infections diagnosed early have been cured by surgery alone.<sup>7</sup> It is mandatory to use amphotericin B treatment if other sites of infections may exist.<sup>2</sup> Reconstructive surgery can be done after cure of the infection.

#### Antifungal treatment

Data on the antifungal susceptibility of mucoraceous fungi to antifungal agents are limited. The correlation between in vitro results and clinical outcome is still controversial for isolates responsible for invasive infections.<sup>94</sup> It is obvious that the in vitro activity of antifungal drugs against the agents of zygomycosis cannot be interpreted by the clinicians if appropriate dosage and extensive surgery have not been prescribed. In fact, despite low minimal inhibitory concentrations recorded in some cases, amphotericin B may still be ineffective,<sup>21,95</sup> and in vitro susceptibility does not necessarily correlate with successful treatment.<sup>56,96</sup> Flucytosine is inactive against this class

of fungi and is not prescribed. Itraconazole has a low activity against zygomycetes, with only 23% of 30 isolates tested inhibited by 1 µg/ml of the drug and 73% by 10 µg/ml,<sup>97</sup> a result confirmed with this drug and other azoles in a few documented cases in which antifungal susceptibility testing was performed.<sup>8,21,56,96</sup>

Thus, despite the fact that new antifungal drugs are now available, amphotericin B (and its lipid formulations) is the drug of choice for the treatment of acute zygomycosis. It is usually prescribed at high doses up to 1.5 mg/kg/day achieved by rapidly escalating doses. Once the patient is stabilized, lower dosages can be given (0.8–1 mg/kg/day) and therapy on alternate days can be instituted.<sup>93</sup> The exact duration and total dose needed are not defined. An optimal treatment requires at least 8–10 weeks until resolution of fever, symptoms, and evidence of infection. The usual total dose is 2–4 g. Although no large clinical trials have been performed, most experts would choose a lipid preparation of amphotericin B over the conventional one. These preparations allow for higher dosing and more prolonged courses of therapy by minimizing the toxicity-limiting events often seen with the conventional form. Experience in zygomycosis is very encouraging as the evidence base continues to build for these compounds.<sup>102,105–107</sup>

One of the latest additions to the antifungal armamentarium in this area is posaconazole. This itraconazole congener is available in oral formulations only and case reports and limited cases series have shown excellent clinical activity against the zygomycetes.<sup>108,109</sup>

#### Complementary procedures

Control of acidosis or hyperglycemia in a diabetic patient is certainly an important contribution to the resolution of the infection. In patients undergoing corticosteroid treatment, discontinuation of the drug, or at least reduction of the dosage, is also recommended. Recovery to a normal granulocyte count, either spontaneously or after injection of hematopoietic growth factors such as granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF), may help in controlling the infection, although the influence of the hematopoietic growth factors on the evolution of documented infections is not obvious so far.<sup>98</sup> In all patients with hematologic malignancies, cure is not achieved without induction of remission. Other approaches have included prescription of gamma interferon and hyperbaric oxygen,<sup>47,99</sup> although there is no evidence that hyperbaric oxygen is useful.<sup>69,93</sup>

#### Prevention of acute zygomycosis

Zygomycosis can develop in patients under empiric amphotericin B therapy, and to date there is no proven regimen effective to prevent acute zygomycosis. However, recent reports of prophylaxis with posaconazole are encouraging.<sup>110–112</sup> Standard procedures of prevention should be applied, such as limiting the sources of contamination in the environment of patients at risk, including those for *Aspergillus* (controlling air-conditioning systems, avoiding construction or renovation work near hematology or transplantation units). Careful monitoring and control of diabetic patients and appropriate use of corticosteroids and deferoxamine should also limit the number of infections. Finally, better training of clinicians and microbiologists should avoid dramatic delays in the diagnosis and treatment of these infections.

## Treatment of chronic zygomycosis

### Surgery

Surgical resection alone is not effective in managing infections caused by *Basidiobolus* or *Conidiobolus* spp. Cosmetic surgery can be proposed after prolonged antifungal therapy and sterilization of the lesion.

### Antifungal therapy

Because of the infrequency of these infections, treatment is not well defined for entomophthoraceous fungi. The dosage, duration, and even the best antifungal drug selection are unclear. Saturated potassium iodide (30 mg/kg/day) has long been the treatment of choice for chronic infections caused by *Basidiobolus* and *Conidiobolus*.<sup>60,67</sup> Since the discovery of azoles, patients have improved with, if not been cured by, ketoconazole or itraconazole,<sup>60,67</sup> whereas recurrence was seen in at least one case.<sup>90</sup> The efficacy of fluconazole ranges from complete cure<sup>61,100</sup> to partial improvement<sup>100</sup> or failure.<sup>90</sup> The role of the newer azoles, such as posaconazole, remains to be determined. Amphotericin B is rarely prescribed for chronic infections.<sup>90</sup>

In the rare cases of disseminated infections caused by *C. incongruus*, the therapeutic approach should probably resemble those for mucoraceous fungi. However, only one of the three patients in one study treated with amphotericin B and surgery survived. In one of the fatal cases, the fungus exhibited in vitro resistance to amphotericin B and flucytosine,<sup>65</sup> although both *Conidiobolus* and *Basidiobolus* are usually susceptible.<sup>101</sup>

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# Hyalohyphomycosis

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## General description

The mycoses encompassed in the hyalohyphomycosis group are very heterogeneous, with only the presence in tissues of hyaline hyphae (without pigment in the wall) as a common characteristic. This term is used as a counterpart to the term “phaeohyphomycosis,” in which fungi appear in tissues as septate but pigmented hyphae. The term “hyalohyphomycosis” is clinically useful when hyaline septate fungi are observed on histopathology without recovery of a pathogen. When the causative agent is recovered (e.g., *Fusarium solani*) a more specific term (fusariosis or infection by *Fusarium* spp.) should be used. By contrast with phaeohyphomycosis, in which four clinical syndromes are well characterized, hyalohyphomycosis does not have any characteristic clinical syndrome.

The number of organisms causing hyalohyphomycosis is increasing and includes *Fusarium* spp., *Penicillium* spp., *Scedosporium brevicaulis*, *Acremonium* spp., and *Paecilomyces* spp.<sup>1-6</sup> Other agents of hyalohyphomycosis include *Aspergillus* spp., *Scopulariopsis* spp., *Beauveria* spp., *Trichoderma* spp., *Chrysosporium* spp., and others (Table 13-1). The disease caused by these pathogens is described in other chapters.

Localized infections may occur among otherwise healthy individuals (usually following penetrating trauma), while disseminated infections tend to occur among severely immunocompromised patients such as those undergoing transplantation (stem cell or organ) and patients with acquired immune deficiency syndrome (AIDS). In the immunosuppressed patient population, the outcome is closely related to the persistence of severe immunosuppression.<sup>7</sup>

## Fusarium

The genus *Fusarium* is a common soil saprophyte and important plant pathogen, which causes a broad spectrum of human disease, including mycotoxicosis, and infections, which can be superficial, locally invasive or disseminated.<sup>8</sup>

The most frequent cause of human infections is *F. solani* but *F. oxysporum*, *F. moniliforme*, *F. proliferatum*, *F. chlamydosporum*,

*F. anthophilum*, *F. dimerum*, *F. sacchari*, and *F. verticillioides* have also been implicated.<sup>9-13</sup>

*Fusarium* species possess several virulence factors, including the ability to produce mycotoxins such as trichothecenes, which suppress humoral and cellular immunity and may also cause tissue breakdown.<sup>14</sup> In addition, *Fusarium* species have the ability to adhere to prosthetic material and to produce proteases and collagenases.<sup>15</sup> *Fusarium solani* is the most virulent species.<sup>16</sup>

## Practical mycology

*Fusarium* spp. grow rapidly on many media (without cycloheximide which is inhibitory). On potato dextrose agar, *Fusarium* spp. produce white, lavender, pink, salmon or gray-colored colonies (which readily change in color) with velvety to cottony surfaces.<sup>17</sup> Microscopically, the hyphae of *Fusarium* in tissue resemble those of *Aspergillus* spp.; the filaments are hyaline, septate, and 3–8 µm in diameter. They typically branch at acute and at right angles. The production of both fusoid macroconidia (hyaline, multicellular clusters, macroconidia with foot cells at the base of the macroconidium) and microconidia (hyaline, unicellular, ovoid to cylindrical in slimy head or chains) are characteristic of the genus *Fusarium* (Fig. 13-1). If microconidia are present, the shape, number of cells (usually 1–3), and mode of cell formation (chains or false heads) are important in identification. Chlamydoconidia are sometimes present and appear singly, in clumps or in chains, and their walls may be rough or smooth.<sup>17</sup>

*Fusarium* can be distinguished from *Acremonium* by its curved, multicellular macroconidia, while *Cylindrocarpon* is distinguished from *Fusarium* by its straight to curved macroconidia which lack foot cells.<sup>18</sup> The identification of *Fusarium* spp. may be difficult and is well described by Nelson et al.<sup>14</sup>

## Epidemiology and clinical spectrum

*Fusarium* species cause a broad spectrum of infections in humans, including superficial, locally invasive, and disseminated infection. The clinical form of fusariosis depends largely on the immune status of the host and the portal of entry of the infection.<sup>8</sup>

**Table 13-1** Hyalohyphomycosis: spectrum of pathogens and infections

Pathogen	Normal host	Immunosuppressed host
<b>More common organisms</b>		
<i>Fusarium</i> spp.	Keratitis Endophthalmitis Bone/joint infection Skin infection Onychomycosis Mycetoma Peritonitis (CAPD)	Mostly disseminated or sinopulmonary infection Brain abscess Skin lesions Peritonitis
<i>Penicillium marneffe</i>	Disseminated infection	Disseminated infection
<i>Scedosporium apiospermum</i>	Keratitis Sinusitis Endophthalmitis Central nervous system infection Bone/joint infection Soft tissue infection Pneumonia Otitis	Disseminated infection Sinusitis Pneumonia Brain abscess and meningitis
<i>Paecilomyces lilacinus</i> , <i>variotii</i>	Sinusitis Keratitis, orbital granuloma Onychomycosis Endocarditis Skin infection Endophthalmitis Peritonitis (CAPD)	Disseminated infection Pyelonephritis Cellulitis Pneumonia
<i>Acremonium</i> spp.	Keratitis Onychomycosis Osteomyelitis, mycetoma Central nervous system infection Endophthalmitis Peritonitis (CAPD) Prosthetic valve endocarditis	Peritonitis Cerebritis Disseminated infection Pneumonia Dialysis-access fistula infection
<i>Scopulariopsis brevicaulis</i>	Keratitis Otomycosis Sinusitis Prosthetic valve endocarditis	Skin lesions Pneumonia
<b>Less common organisms</b>		
<i>Beauveria</i> spp.	Keratitis	Not described
<i>Chaetoconidium</i> spp.	Skin lesions	Skin lesions
<i>Chrysosporium</i> spp.	Keratitis Osteomyelitis Endocarditis	Disseminated infection Sinusitis

(Continued)



**Table 13-1** Hyalohyphomycosis: spectrum of pathogens and infections—cont'd

Pathogen	Normal host	Immunosuppressed host
<i>Myriodontium keratinophilum</i>	Sinusitis	Not described
<i>Neurospora sitophila</i>	Endophthalmitis	Not described
<i>Trichoderma</i> spp.	Peritonitis (CAPD) Pulmonary fungus ball	Pneumonia Disseminated infection

CAPD, continuous ambulatory peritoneal dialysis.  
*Scedosporium apiospermum* produces hyaline moulds unlike *Scedosporium prolificans* (also known as *S. inflatum*) which produces dark pigments and is classified among the agents of phaeohyphomycosis.  
 Some *Scopulariopsis* spp. produces dark pigments and are classified among the agents of phaeohyphomycosis.

**Figure 13-1** Microscopic appearance of *Fusarium* spp., showing the typical banana-shaped macroconidia (courtesy of [www.doctorfungus.org](http://www.doctorfungus.org) © 2007).

Among immunocompetent hosts, keratitis and onychomycosis are the most common infections. Less frequently, the infection may occur as a result of skin breakdown, such as burns and wounds,<sup>19</sup> or the presence of foreign bodies, such as keratitis in contact lens wearers<sup>20</sup> at times causing outbreaks of fusarial keratitis.<sup>21</sup> Peritonitis in patients receiving continuous ambulatory peritoneal dialysis has also been described.<sup>22-24</sup> Other infections in immunocompetent patients include sinusitis,<sup>25</sup> pneumonia,<sup>26,27</sup> thrombophlebitis,<sup>28</sup> fungemia with or without organ involvement,<sup>19,29</sup> endophthalmitis,<sup>30,31</sup> septic arthritis,<sup>32</sup> and osteomyelitis.<sup>33</sup> Two outbreaks of fusarial keratitis were recently described in the United States (164 cases) and Singapore (66 cases). Case-control studies in the two populations of patients showed that keratitis was more likely to occur in patients who used a specific contact lens solution (ReNu with MoistureLoc).<sup>34,35</sup>

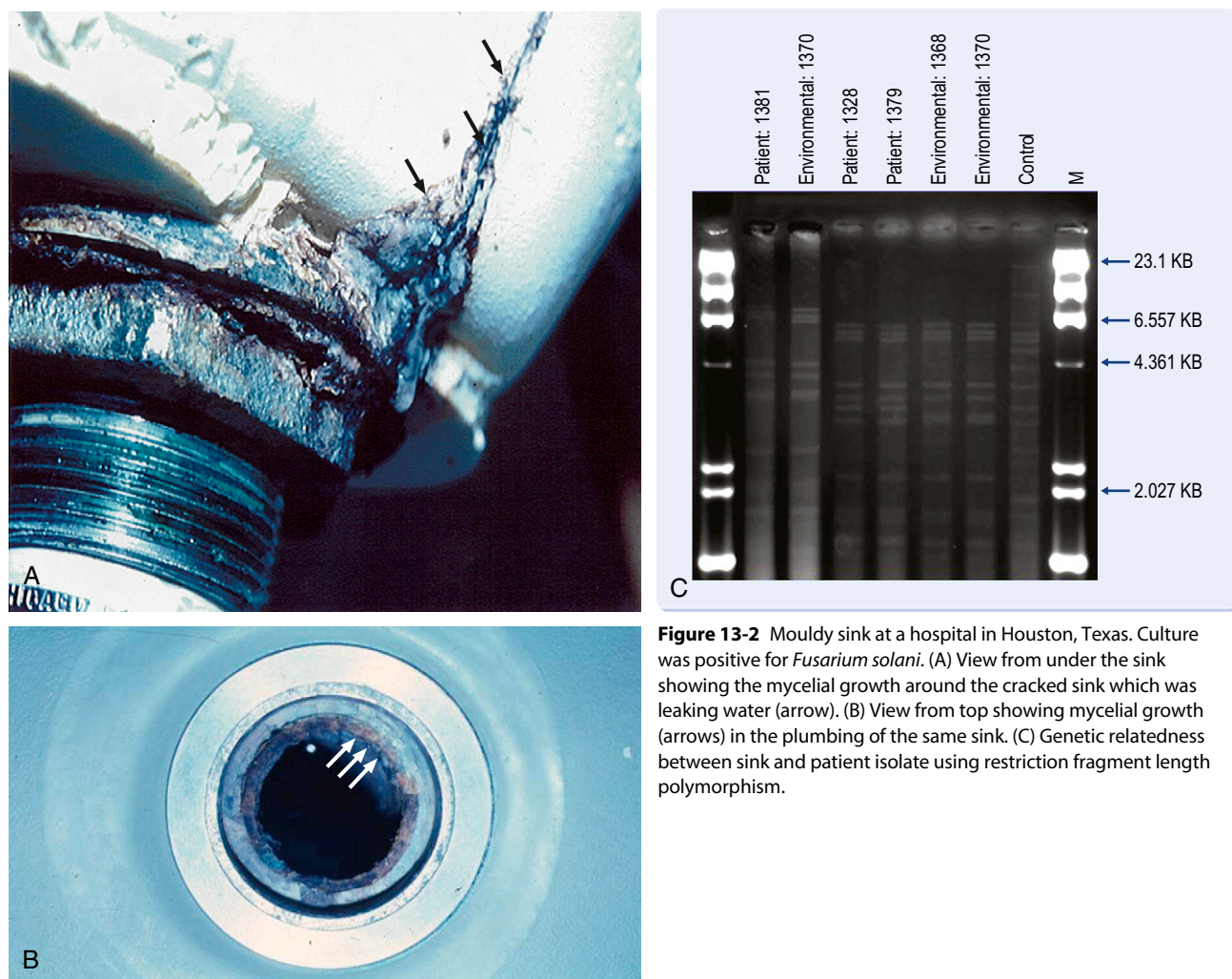
Immunocompromised patients at high risk for fusariosis are those with prolonged and profound neutropenia and/or severe T cell immunodeficiency.<sup>10</sup> Unlike infection in the normal host, fusariosis in the immunocompromised population is typically invasive and disseminated.<sup>19</sup> In patients with hematologic diseases, the infection occurs more frequently in neutropenic patients with acute leukemia.<sup>7</sup> In the allogeneic hematopoietic stem cell transplant (HSCT) population, the infection has a trimodal distribution with a first peak in the early posttransplant period (during neutropenia), followed by

a peak at a median of 70 days after transplant among patients with acute graft-versus-host disease (GvHD) receiving corticosteroids, and a third peak >1 year after transplant during treatment for chronic extensive GvHD. Severe T cell immunodeficiency and not neutropenia is the major risk factor for fusariosis in these patients.<sup>36</sup> The overall incidence of fusariosis is ~6 cases per 1000 HSCT: lowest (~1.5–2/1000) among autologous recipients, intermediate (~2.5–5/1000) in matched related and matched unrelated allogeneic recipients, and highest (20/1000) among recipients of mismatched related donor allogeneic HSCT.<sup>36</sup> Locally invasive and usually late infections may also develop among solid organ transplant (SOT) recipients,<sup>37</sup> but appear to be less common than among HSCT patients.

The portals of entry include the paranasal sinuses,<sup>38,39</sup> lungs,<sup>40,41</sup> and skin.<sup>10,19</sup> Airborne fusariosis is thought to be acquired by the inhalation of airborne *Fusarium* conidia, as suggested by the occurrence of sinusitis and/or pneumonia in the absence of dissemination. The role of skin as a portal of entry is supported by the development of infection following skin breakdowns due to trauma (automobile accidents, bamboo), burns or onychomycosis in normal hosts,<sup>19</sup> and the development of cellulitis (typically at sites of tissue breakdown such as toes and fingers) which may remain localized or lead to disseminated infection in immunocompromised patients.<sup>7,10</sup>

Given the ubiquity of *Fusarium* species in the environment, fusariosis may potentially be acquired in the community, as suggested by the presence of airborne fusarial conidia in outdoor air samples.<sup>10,42,43</sup> In a prospective study, *Fusarium* species were recovered from a hospital water system (water, water storage tanks, shower and sink drains, shower heads and sink faucet aerators) and from hospital air and other environments (Fig. 13-2).<sup>43</sup> *Fusarium* species were also present in the outdoor air. Showering and other water-related activities appeared to be an efficient mechanism for the dispersal of airborne fusarial conidia and transmission to the immunocompromised host, as shown by the close molecular relatedness between water and patients' isolates.

The genetic diversity of patients' and environmental isolates of *F. oxysporum* recovered from three locations in the United States was recently studied. Results indicated that a geographically widespread clonal lineage was responsible for >70% of all clinical isolates, and strains of this clonal lineage were genetically similar to those isolated from the water system of three US hospitals,<sup>44</sup> further supporting the risk of nosocomial waterborne fusariosis.



**Figure 13-2** Mouldy sink at a hospital in Houston, Texas. Culture was positive for *Fusarium solani*. (A) View from under the sink showing the mycelial growth around the cracked sink which was leaking water (arrow). (B) View from top showing mycelial growth (arrows) in the plumbing of the same sink. (C) Genetic relatedness between sink and patient isolate using restriction fragment length polymorphism.

## Clinical presentation

### Normal host

*Fusarium* spp. may cause localized infections of the cornea (Fig. 13-3), skin, and nails in the normal host. Fusarial keratomycosis is usually the result of several factors: trauma and penetration of the cornea by soil or plant material, lack of hygiene resulting in contamination of soft contact lenses, and local immunosuppression due to corticosteroid eye drops. Onychomycosis can also be caused by *Fusarium* spp. The typical clinical presentation is that of a distal subungual lesion in the toenails of females.<sup>45</sup> *Fusarium* spp. may also cause superficial infections typical of dermatophytes, such as intertrigo,<sup>45</sup> tinea pedis and hyperkeratotic plantar lesions.<sup>46</sup> In addition, *Fusarium* spp. have been increasingly reported as a cause of non-dermatophyte skin infections.<sup>47</sup> Other fusarial infections in normal hosts include surgical wound infections, ulcers and otitis media.<sup>14,48</sup>

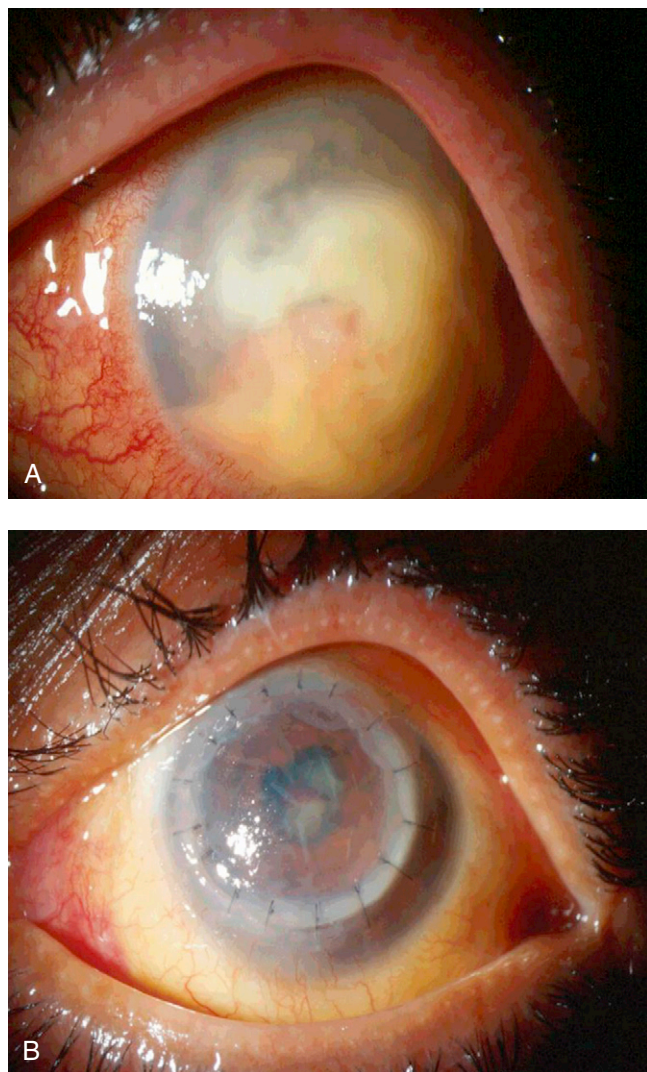
Localized deep *Fusarium* infections are rare in non-immunosuppressed individuals and occur following direct inoculation of various body sites. The different infections such as endophthalmitis, osteomyelitis, septic arthritis, pneumonia, brain abscess, cystitis, peritonitis and subcutaneous infections do not have a typical pattern suggestive of fusariosis.

### Immunosuppressed host

The most common presentation of fusarial infection in immunosuppressed patients is persistent fever refractory to antibacterial and antifungal therapy. Other findings at presentation include sinusitis and/or rhinocerebral infection, cellulitis at the site of skin breakdown, endophthalmitis, painful skin lesions (Fig. 13-4), pneumonia, myositis, and infections of the central nervous system.<sup>3,8,38</sup> Three types of cutaneous lesions can be observed: ecthyma-like lesions, target lesions consisting of the ecthyma-like lesions surrounded by a thin rim of erythema (rare), and multiple subcutaneous nodules, at times painful. It is possible that these cutaneous lesions represent, in fact, an evolution of the same lesions observed at different ages.<sup>19</sup> In primary fusarial pneumonia, symptoms of pleuritic chest pain, fever, cough, and hemoptysis indistinguishable from pulmonary aspergillosis characterize the disease.<sup>3,10</sup>

The features of patients with disseminated infection are similar in many respects to those of patients with disseminated aspergillosis.<sup>3,10</sup> Unlike aspergillosis, however, infection with *Fusarium* spp. is associated with a high incidence of skin and subcutaneous lesions and positive blood cultures.<sup>3,10</sup>





**Figure 13-3** *Fusarium* fungal keratitis: (A) before treatment; (B) after resolution (courtesy of Richard Graybill MD).

Overall mortality of fusarial infections in immunocompromised patients ranges from 50% to 90%.<sup>10</sup> Persistence of severe immunosuppression is the most important factor related to poor outcome.<sup>7,36</sup>

Among patients undergoing SOT, fusarial infections tend to be more localized, occur later after transplantation and have a better outcome than among patients with hematologic cancer or recipients of bone marrow transplant (BMT).<sup>37</sup>

## Diagnosis

The diagnosis of fusariosis depends on the clinical form of the disease. The clinical picture is not of help in the diagnosis of keratitis, since the clinical manifestations are similar regardless of etiology (bacteria, fungi). Culture of corneal scrapings (most frequent) or tissue biopsy is usually required for a definitive diagnosis.

In patients with severe immunosuppression, the growth of a mould from the bloodstream and/or the presence of preceding or concomitant toe or finger cellulitis (Fig. 13-5) or cutaneous

or subcutaneous lesions should raise the suspicion of fusarial infection.<sup>3,10</sup>

The radiologic findings of pulmonary fusarial infection range from non-specific infiltrates (most commonly) to nodular and/or cavitary lesions, depending on the timing of the study.<sup>3</sup>

The definitive diagnosis requires the isolation of *Fusarium* spp. from clinical specimens (blood, skin, sinuses, lungs, other). Culture identification is important because of the histopathologic similarities between *Fusarium*, *Aspergillus*, and other similar fungi. Like *Aspergillus* spp., *Fusarium* spp. invade blood vessels, causing thrombosis and tissue infarction, and appear in tissues as acute branching septate hyphae.<sup>17</sup> However, sporulation may be present in tissue,<sup>49</sup> and the finding of hyphae and yeast-like structures together is highly suggestive of fusariosis in the high-risk population. In the absence of microbial growth, distinguishing fusariosis from other hyalohyphomycoses may be difficult, and requires the use of in situ hybridization in paraffin-embedded tissue specimens.<sup>50</sup> Although the genus *Fusarium* can be identified by the production of hyaline, curved, multicellular macroconidia with a foot cell, species identification is difficult and may require molecular methods. More recently, a commercially available PCR-based method was tested in 21 clinical isolates of *Fusarium* species and 5 ATCC isolates. Using sequencing identification as a gold standard, 7/9 different species were identified.<sup>51</sup>

The  $\beta$ 1,3-D-glucan test is usually positive in invasive fusarial infections but cannot distinguish *Fusarium* from other fungal infections (*Candida*, *Aspergillus*, *Trichosporon* and others) which are also detected by the assay.<sup>52,53</sup> However, a positive  $\beta$ 1,3-D-glucan test and a negative galactomannan test in a high-risk patient with mould infection is highly suggestive of fusariosis.

## Prevention and Treatment

Because of the poor prognosis associated with fusariosis and the limited susceptibility of *Fusarium* spp. to antifungal agents, prevention of infection remains the cornerstone of management. In severely immunocompromised patients, every effort should be made to prevent patient exposure (e.g., by putting high-risk patients in rooms with an HEPA filter and positive pressure, avoiding contact with reservoirs of *Fusarium* spp., such as tap water,<sup>43</sup> and/or cleaning showers prior to use by high-risk patients).<sup>54</sup>

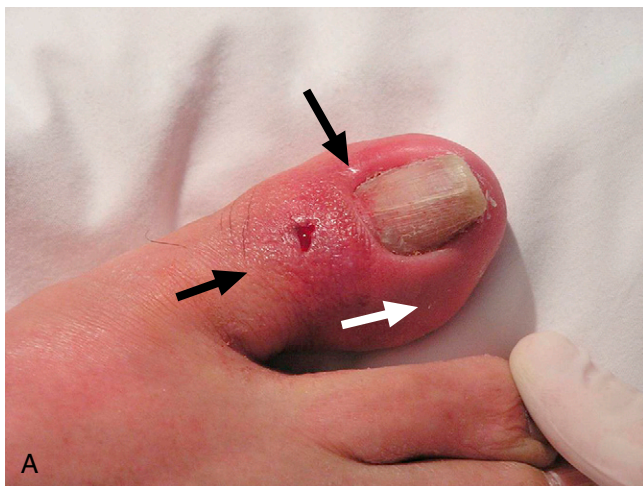
Decreasing immunosuppression should be attempted in patients with prior history of *Fusarium* infection and can be achieved by a reduction in or discontinuation of immunosuppressive agents, shortening the duration of neutropenia (selection of non-myeloablative as opposed to myeloablative preparative regimens for allogeneic HSCT and the use of preemptive G-CSF or GM-CSF and dexamethasone-elicited white blood cell transfusions).<sup>55,56</sup> If the organism is available, antifungal susceptibility testing should be performed and antifungal prophylaxis with an agent active against the recovered fusarial strain should be considered. In addition, a thorough evaluation and treatment of skin lesions (particularly onychomycosis that serve as a portal of entry for *Fusarium*) should be done prior to commencing antineoplastic therapy.<sup>19</sup>

The skin may be the primary source of these life-threatening infections, usually at the site of preexisting onychomycosis or skin breakdown from a local infection, and typically presents



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**Figure 13-4** Metastatic (secondary) skin lesions in fusariosis. (A) Papular, erythematous-violaceous lesions of disseminated fusariosis in a leukemic patient. Skin lesions in disseminated disease are papular, nodular, and painful. Central necrosis is frequent, giving the appearance of ecthyma gangrenosum. (B) Skin lesions at different sizes and ages: papulonodular lesions; one has progressed to ecthyma gangrenosum (*large arrows*); occasionally, a target lesion is formed, with a thin rim of erythema surrounding the papular or nodular lesions (*smaller, thin arrows*). (C) Skin lesions are usually multiple. (D) Bullae may rarely be seen (reprinted with permission from Nucci and Anaissie. Clin Infect Dis 35:909, 2002).



**Figure 13-5** Primary skin lesions in fusariosis. (A) Fusarial onychomycosis with periungual cellulitis spreading on the dorsum of the foot (*arrows*). (courtesy of Maria-Cecilia Dignani MD) (B) Cellulitis in the dorsum of the foot secondary to interdigital fusarial infection in a neutropenic patient with multiple myeloma after autologous HSCT. Note lymphangitic spread (*arrows*).

as cellulitis in the severely immunocompromised patient (see Fig. 13-5), later spreading to cause disseminated disease. Hence, we recommend that patients with hematologic cancer who have onychomycosis or primary skin lesions following a trauma or a bite, such as a spider bite, and who are about to undergo cytotoxic chemotherapy and/or HSCT be evaluated by a dermatologist to ascertain the nature of their onychomycosis or skin breakdown and rule out the presence of fusarial infection. In the presence of tissue breakdown, we also recommend that these patients avoid contact of the damaged tissue with tap water (usually contaminated with pathogenic moulds).

Early therapy of localized disease (when present) is important to prevent progression to a more aggressive or disseminated infection. This therapy should include surgical debridement, topical natamycin, and probably systemic antifungal chemotherapy (see section on Treatment).<sup>10,41,57</sup> Because of the risk of relapse in immunosuppressed patients with prior fusarial infections,<sup>39</sup> secondary prophylaxis should be considered (IV amphotericin B or its lipid formulation, itraconazole, voriconazole, posaconazole). In addition, consideration should be given to postponing cytotoxic therapy or using prophylactic G-CSF or GM-CSF stimulated granulocyte transfusions if delay in treating the underlying cancer is not possible.<sup>10,55,58</sup>

## Penicillium

*Penicillium marneffei* is the only *Penicillium* species (among more than 200) to cause significant human disease in relative healthy individuals. This thermally dimorphic organism is restricted to Asia (Southeast and Far East) where it is considered an indicator for AIDS. Regions reported to be endemic for *P. marneffei* infections include Indonesia, Laos, Hong Kong, Singapore, Thailand, Myanmar, Malaysia, Vietnam, Taiwan, and the Guangxi province of China.<sup>6,59</sup> However, due to intensive migration, cases of infection due to *P. marneffei* outside this geographic area have been reported in people who traveled to these regions.<sup>60,61</sup> This infection is the third most common opportunistic infection, after tuberculosis and cryptococcosis, in HIV-infected individuals who live in endemic regions.<sup>62</sup>

No definite route of transmission has been established, although the known natural carrier for the organism is the bamboo rat, and molecular studies have shown that humans and bamboo rats share genetically identical isolates.<sup>63</sup> The fungus may have a prolonged latency period. In one case, symptomatic infection developed 10 years after travel to Southeast Asia.<sup>64</sup>

*Penicillium* spp. other than *P. marneffei* can rarely cause disease among immunocompromised and immunocompetent hosts.<sup>65,66</sup>

## Practical mycology

*Penicillium* spp. grow at 25°C on Sabouraud dextrose agar, Czapek agar and other mycologic media that lack cycloheximide. Colonies are initially white, change to a brownish red color and later to green or bluish green color. The colony surface appears flat and powdery.<sup>67</sup>

*Penicillium marneffei* should be incubated at 30°C for 2 weeks to display dimorphism. The yeast phase (37°C) displays colonies that are white to tan, soft, and dry. Microscopically,

the organism grows as a single yeast-like cell and reproduces by fission rather than budding. The round or oval or sometimes elongate cells (approximate diameter 3 µm) are septate. Elongated and septate allantoid forms (length 8–13 µm) and short filaments may also be present. The most distinguishing characteristic of the mould phase (at 25°C) is the early presence of a red pigment that diffuses into the agar. The colonies start as pinkish-yellow and evolve into a bluish-green color in the center with a white periphery. *P. marneffei* displays the characteristic brush-like conidia with terminal conidiophores that bear groups of 4–5 metulae supporting groups of 4–6 phialides (Figs 13-6 and 13-7).<sup>67</sup>

*Penicillium* is differentiated from *Scopulariopsis* which forms annelides having annelloconidia with truncate bases and *Paecilomyces* which forms phialides having long, tapering apices.<sup>67</sup>

## Incidence

The incidence of *P. marneffei* infections, in both travelers and residents of endemic areas, has seen a dramatic rise as a result of the AIDS epidemic (approximately 25% of the AIDS patients living in Thailand are affected by this infection),<sup>68</sup> but with the reduction in transmission of HIV, concomitant decreases in the incidence of *P. marneffei* infection have been observed.<sup>6</sup> Penicilliosis has also been reported in healthy as well as immunocompromised children and adults.<sup>59</sup> No seasonal variation in the incidence of penicilliosis has been reported, except for one report suggesting a higher incidence during the rainy season in northern Thailand.<sup>69</sup>

## Risk factors

The major risk factors for the acquisition of infection are travel to or residence in endemic areas and severe immunosuppression secondary to AIDS or other conditions such as organ or stem cell transplantation, lymphoproliferative disorders, and corticosteroid therapy.<sup>6</sup>



**Figure 13-6** Microscopic morphology of *P. marneffei* showing hyaline, smooth-walled conidiophores bearing terminal verticils of 3–5 metulae, each bearing 3–7 phialides. Conidia are globose to subglobose, 2–3 µm in diameter, smooth-walled and are produced in basipetal succession from the phialides (courtesy of [www.doctorfungus.org](http://www.doctorfungus.org) © 2007).

## Clinical presentation

The lungs are the usual initial site of infection and the clinical manifestations are non-specific.<sup>70</sup> Most affected individuals present with widespread infection closely resembling acute disseminated histoplasmosis.<sup>71-73</sup>

Disseminated infection usually presents with fever, marked weight loss, anemia, leukocytosis or leukopenia, generalized papular skin lesions (60–70%), cough (50%), lymphadenopathy, and hepatosplenomegaly and may rapidly progress to death if untreated.<sup>6,59,62,74</sup> Other cutaneous manifestations include necrotic papules, rash, acne-like pustules, and/or nodules and occur more commonly on the face, upper trunk, and extremities (Fig. 13-7). Molluscum contagiosum-like lesions tend to occur more commonly in HIV-infected patients and involve the palatal and pharyngeal regions.<sup>75</sup> Other organs may be involved, including bone marrow, bowels, kidneys, pericardium, meninges, and others. A high index of suspicion should be maintained when a susceptible patient has papular molluscum contagiosum-like skin lesions and a non-specific febrile illness.<sup>76</sup>

## Diagnosis

A history of travel to an endemic area is of paramount importance. A rapid presumptive diagnosis can be made by microscopic examination using Giemsa, Wright stain, Gomori Methenamine silver (GMS) or periodic acid-Schiff (PAS) on various specimens (see Fig. 13-7) (bone marrow, peripheral blood, and skin fluid). This microscopic examination will show the characteristic intracellular, septate, yeast-like cells. The diagnosis is confirmed by culture. Of note, the lysis centrifugation blood culturing method is very effective at recovering *P. marneffeii*.

The radiologic findings in pulmonary penicilliosis appear as reticulonodular, nodular, diffuse alveolar infiltrates and/or rarely cavitory associated with hemoptysis.<sup>70,77</sup>

Histopathologic findings depend on the patient's immune status: granulomatous or suppurative in relatively immunocompetent patients, and necrotizing in severely immunocompromised hosts. The granulomatous reaction is usually found in the organs of the reticuloendothelial system, where histiocytes, lymphocytes, epithelioid plasma cells, and occasionally giant cells form the granuloma. As the histiocytic granulomas expand, releasing fungal cells and accumulating neutrophils, central abscesses eventually form. In immunosuppressed patients, necrotic lesions are characterized by focal necrosis surrounded by histiocytes engorged by the proliferating fungal cells. In all these histopathologic reactions, microscopic examination reveals yeast cells both within phagocytes (resembling *H. capsulatum* var. *capsulatum*) and extracellularly (in which yeasts appear larger than the intracellular form) (see Fig. 13-7).<sup>71</sup>

Various tests based on antigen and antibody detection and PCR-based methods have been developed for the diagnosis of *P. marneffeii* infection. In general, these tests have good sensitivity and specificity.<sup>6,78</sup>

## Secondary prevention

Secondary prophylaxis with itraconazole 200 mg/day is indicated in HIV-infected patients with history of *P. marneffeii* infection.<sup>62</sup> Similar to other mycoses, discontinuation of prophylaxis after the introduction of highly efficient antiretroviral therapy is feasible.<sup>79</sup>

## Scedosporium

*Scedosporium* spp. are commonly isolated from rural soils, polluted waters, composts, and from manure of cattle and fowl. Infections are caused by two species: (1) *Pseudallescheria boydii* (perfect state) and its *S. Apiospermum* (imperfect state) and (2) *S. prolificans* (syn *S. inflatum*) and which is classified as an agent of phaeohyphomycosis. Two forms of disease have been described: invasive tissue disease (both agents) and mycetoma (only *P. boydii*) (see Chapters 14 and 24).<sup>80-82</sup>

## Practical mycology

On Sabouraud dextrose agar, the colonies grow rapidly, producing a white fluffy or tufted aerial mycelium, which later turns to a brownish gray color.<sup>83</sup>

Microscopically, the hyphae of *P. boydii* are hyaline. The conidia are borne singly or in small groups on elongate, simple or branched annellides or laterally from intercalary annellides within the hyphae (Fig. 13-8). *Scedosporium prolificans* can be differentiated from *P. boydii* by its swollen conidiogenous cells. In addition, the growth of *S. prolificans* is inhibited by cycloheximide in Mycosel agar.<sup>83</sup> Unlike *Sporothrix schenckii* and *Blasatomyces dermatitidis*, *Scedosporium* spp. are not dimorphic.

## Incidence

Serious *Scedosporium* infections have increased in the past few years among patients with hematologic malignancies, particularly those undergoing allogeneic HSCT.<sup>2,82</sup> These infections have also been reported to occur in patients with AIDS, SOT, and in patients with cystic fibrosis.<sup>84-86</sup>

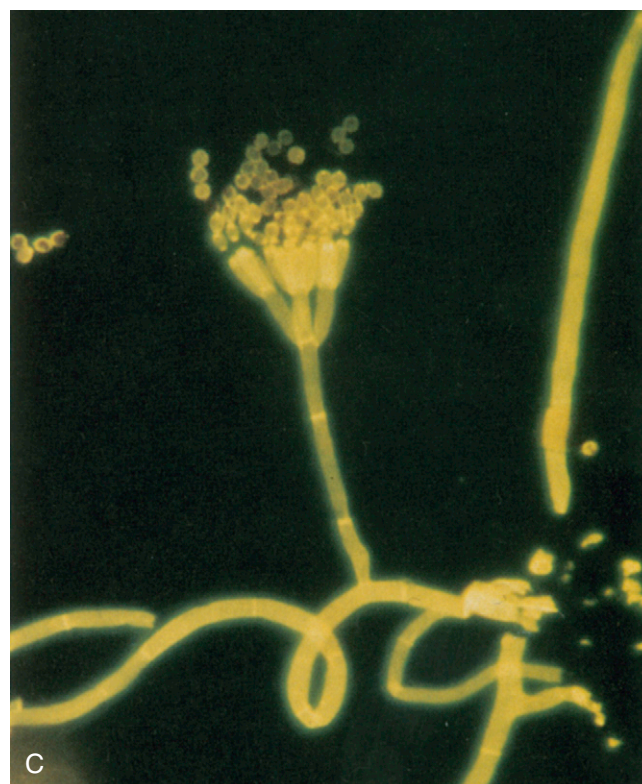
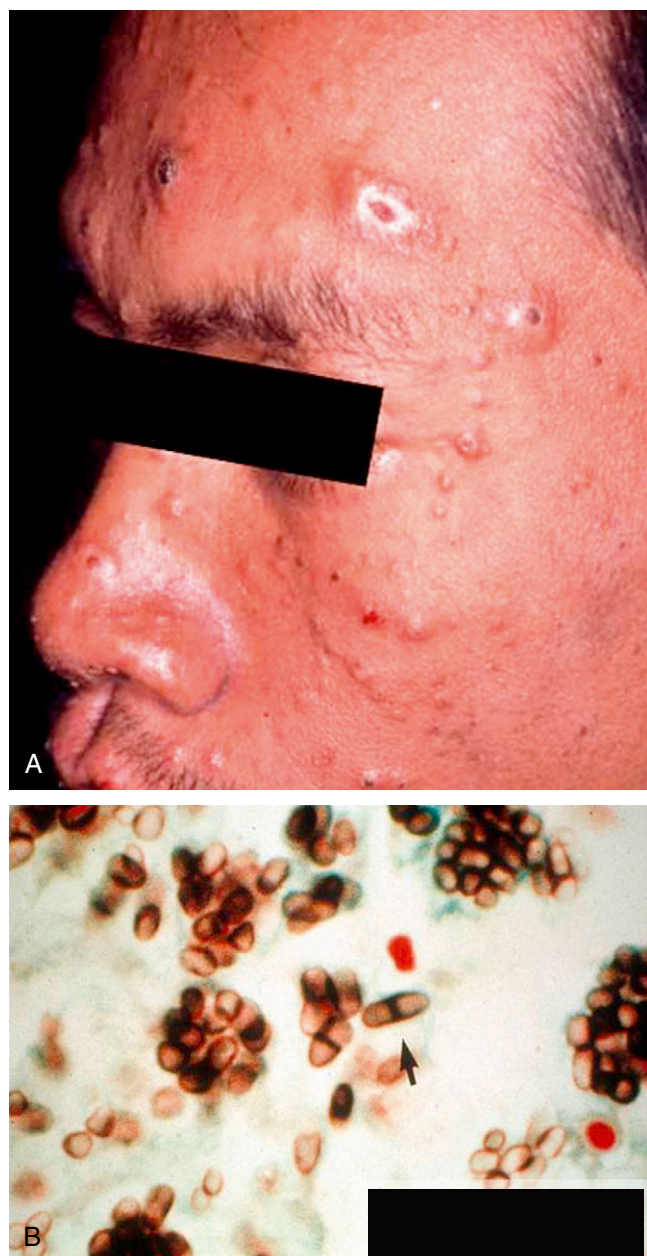
## Clinical presentation

Infection by *Scedosporium* spp. may be secondary to inoculation of fungi after local trauma among otherwise healthy individuals, inhalation of fungal spores, ingestion of contaminated food, and with no apparent source.<sup>84</sup> The clinical spectrum of infection in immunocompetent hosts includes keratitis, endophthalmitis, otitis, sinusitis, central nervous system infections, osteoarticular and soft tissue infections, and pneumonia after near drowning.<sup>87-93</sup> In the setting of severe immunosuppression, deep-seated infections can particularly involve any organ with a predilection for skin (painful cutaneous nodules which may later become necrotic), sinuses, lungs, and central nervous system.<sup>2,80,85,88,94-104</sup>

In healthy individuals cerebral infection is secondary to contiguous spread from sinusitis,<sup>105</sup> penetrating trauma<sup>106</sup> or following near drowning in polluted water.<sup>90,107</sup> In immunocompromised patients, central nervous system infections tend to occur following hematogenous dissemination.<sup>88,95,97,108,109</sup> The majority of cerebral infections have presented as a brain abscess but ventriculitis and meningitis have also been reported.<sup>106,109-111</sup> Delayed treatment of brain abscesses due to *P. boydii* is associated with a high mortality rate (>75%).<sup>106,112</sup>

*Pseudallescheria boydii* can grow within poorly draining bronchi, lung cavity or paranasal sinuses without causing invasive disease,<sup>113</sup> where the fungus ball is the only significant consequence of fungal colonization.<sup>114</sup> Allergic bronchopulmonary disease has also been attributed to *P. boydii*.<sup>115,116</sup>





**Figure 13-7** Penicilliosis (*P. marneffei*). (A) Cutaneous lesions resulted from the dissemination of the fungus from the lungs. The patient's underlying disease is AIDS (B) A Giemsa-stained touch smear showing the typical septate yeast-like cells of *P. marneffei* that reproduce by fission. (C) Fruiting head of *Penicillium* spp. showing a penicillus. The penicillus measures 100–250  $\mu\text{m}$  and consists of phialides and metulae that extend directly from the conidiophore (courtesy of [www.doctorfungus.org](http://www.doctorfungus.org) © 2007). (Reproduced with permission from De la Maza LM, Pezzlo MT, Baron EJ. Color Atlas of Diagnostic Microbiology. Mosby, St Louis, 1997).

## Diagnosis

The radiographic findings of pulmonary infections show areas of nodularity, alveolar infiltrates or, most commonly, consolidation, which may evolve to cavitation.<sup>117-119</sup>

Identification of the fungus by culture is important because of the variable susceptibility of these fungi to amphotericin B and other antifungal agents. The organisms may be recovered in sterile fluid (rarely from blood) and from infected organs. Histopathologic findings are similar to those of aspergillosis, with the presence of acute branching hyphae, blood vessel invasion and thrombosis.<sup>104,120,121</sup>

## Paecilomyces

*Paecilomyces* spp. are isolated from soil and decaying plant material, and often associated with decay of food products and cosmetics.

## Practical mycology

*Paecilomyces* spp. grow rapidly on Sabouraud dextrose agar without cycloheximide. The colonies are at first floccose and white, then change color; the texture is wooly to powdery.



**Figure 13-8** *Scedosporium apiospermum*. One-celled conidia developing from annellides. Phase contrast microscopy, 630× *Scedosporium prolificans* is distinguished from *S. apiospermum* by having basally swollen (inflated), flask-shaped annellides, slower colony development on nutrient agar media, and by not growing on media containing cycloheximide (actidione). (Courtesy of [www.doctorfungus.org](http://www.doctorfungus.org) © 2007).

Colonies of *P. variotii* are velvety and tan to olive-brown in color, while those of *P. lilacinus* are pink or vinaceous to lilac.<sup>122</sup> Microscopically, the *Paecilomyces* spp. conidia are unicellular, ovoid or fusoid, and form chains that can be intertangled. Phialides have a swollen base and a long tapered neck (Fig. 13-9).

## Clinical presentation

The two most common species of *Paecilomyces*, *P. lilacinus* and *variotii*, are rarely pathogenic in humans. In normal hosts, these organisms have been implicated as etiologic agents of keratitis associated with corneal implants, endophthalmitis, endocarditis following valve replacement, sinusitis, and peritonitis in dialysis patients, and cutaneous infections.<sup>123-128</sup> Disseminated infection, pneumonia, cellulitis, fungemia and pyelonephritis have been reported in immunosuppressed patients.<sup>129-134</sup> The portal of entry involves breakdown of skin or mucous membranes and inhalation.<sup>5</sup> Infections associated with contamination of fluids and air conditioning systems have been reported.<sup>126,135,136</sup>

## Acremonium (Cephalosporium)

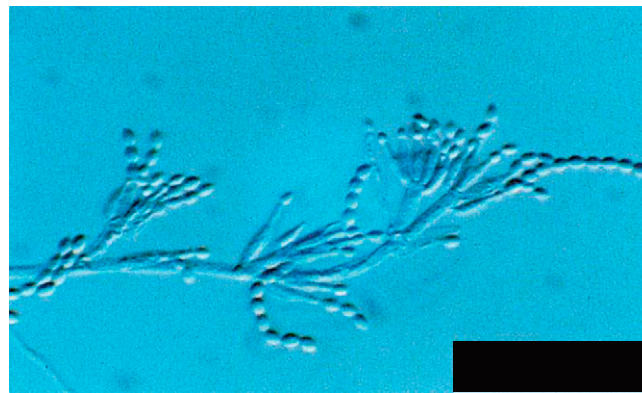
Species of *Acremonium* are commonly found in soil, decaying vegetation, and decaying food.

## Practical mycology

*Acremonium* spp. have moderate growth on Sabouraud agar without cycloheximide. The colonies are white-gray or rose in color, with a velvety to cottony surface.<sup>137</sup> The conidia may be single-celled, in chains or in conidial masses, arising from short, unbranched, single, tapered phialides (Fig. 13-10).<sup>137</sup>

## Clinical presentation

Species reported to cause infections in humans include *A. alabamensis*, *A. falciforme*, *A. kiliense*, *A. roseogriseum*, *A. strictum*, *A. potroni*, and *A. recifei*. This genus has long



**Figure 13-9** *Paecilomyces lilacinus*. Conidiophores and conidia. Branching conidiophores with groups of phialides having characteristic long, tapering, conidia-bearing apices. Conidia in chains are elliptical (courtesy of [www.doctorfungus.org](http://www.doctorfungus.org) © 2007).

been recognized as an etiologic agent of nail and corneal infection, mycetoma, peritonitis and dialysis fistulae infection, osteomyelitis, meningitis following spinal anesthesia in a normal person, cerebritis in an intravenous drug abuser, endocarditis in a prosthetic valve operation, and a pulmonary infection in a child. Occasional deep *Acremonium* infections have been reported in patients with serious underlying medical conditions.<sup>1</sup>

## Scopulariopsis brevicaulis

*Scopulariopsis* spp. are frequently isolated from soil.

## Practical mycology

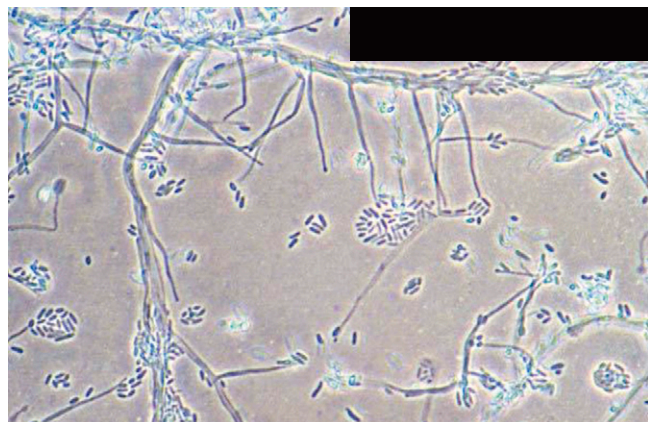
The most common species are *S. brevicaulis* and *S. brumptii*. *Scopulariopsis brevicaulis* produces rather rapidly growing colonies that are powdery, and tan to beige. The reverse side of the colony is usually tan with a brown center. Microscopically, the conidiogenous cells (annellides) are produced from unbranched or branched penicillate-like conidiophores. Conidia are in chains with the youngest conidium at the base of the chain next to the tip of the annellide. The conidia are thick-walled, round to lemon-shaped, rough to spiny with hyaline or brown cell walls (Fig. 13-11).

*Scopulariopsis* can be distinguished from *Penicillium* by their pyriform annelloconidia, typically with truncate bases. *Penicillium* forms globose to subglobose phialoconidia.

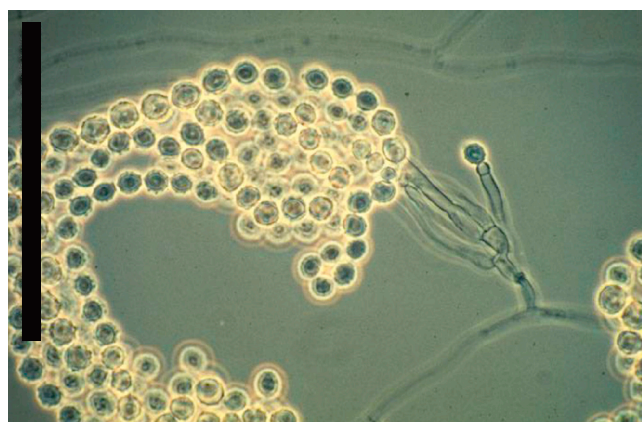
## Clinical presentation

*Scopulariopsis brevicaulis* rarely causes human infection. In healthy individuals this organism has been reported to cause onychomycosis,<sup>138,139</sup> keratitis,<sup>140</sup> otomycosis,<sup>141</sup> invasive sinusitis,<sup>142</sup> and prosthetic valve endocarditis.<sup>143,144</sup> Invasive infections have been reported among immunocompromised patients (recipients of liver transplantation and patients with hematologic malignancies). These infections involved mainly soft tissues and lungs.<sup>145-152</sup>





**Figure 13-10** Microscopic morphology of an *Acremonium* sp. showing long, hyaline, awl-shaped, simple, erect, phialides arising from hyphae or fascicles. Conidia are usually one-celled (ameroconidia), hyaline, globose to cylindrical, and mostly aggregated in slimy heads at the apex of each phialide (courtesy of [www.doctorfungus.org](http://www.doctorfungus.org) © 2007).



**Figure 13-11** *Scopulariopsis brevicaulis*. Septate mycelium, with single, unbranched conidiophores or branched "penicillus-like" conidiophores. Anellides produce chains of lemon-shaped conidia (annelloconidia) with a rounded tip and truncate base. Potato glucose agar, 30°C, phase-contrast microscopy (courtesy of [www.doctorfungus.org](http://www.doctorfungus.org) © 2007).

## Other pathogens

Other rare pathogens known to cause opportunistic hyalohyphomycosis include the following.

*Aphanoascus fulvescens* (syn. *Anixiopsis fluvescens* var. *stercoraria*, *Anixiopsis fluvescens*), the teleomorph of *Chrysosporium keratinophilum* may cause an infection that resembles a dermatophyte infection. This keratinophilic fungus is found in soil.<sup>153</sup>

*Arthrographis kalrae* (*Oidiendron kalrai*) is a fungus found in soil. It was reported to cause invasive pansinusitis with central nervous system involvement in an AIDS patient, mycetoma in a healthy individual, and keratitis in a contact lens wearer.<sup>154-157</sup>

*Beauveria* spp. can cause keratitis following invasive procedures on the eye.<sup>158-160</sup> The management of this infection with medical treatment is usually unsuccessful and it requires surgery.

*Chaetoconidium* spp. have been cultured from biopsy specimens of a skin lesion in a renal transplant patient treated with immunosuppressive therapy.<sup>161</sup>

*Chrysosporium* spp. have been reported to cause disseminated disease<sup>162-164</sup> and invasive sinusitis<sup>165</sup> among immunocompromised hosts. In healthy individuals these organisms may cause keratitis,<sup>166</sup> pulmonary granulomas,<sup>167</sup> endocarditis,<sup>168</sup> and osteomyelitis.<sup>169</sup> Amphotericin B and liposomal amphotericin B (Ambisome) have been associated with successful treatment,<sup>162,169</sup> while itraconazole was associated with relapse in one case report.<sup>162</sup>

*Myriodontium keratinophilum* has been isolated from a frontal sinusitis secondary to nasal polyps.<sup>170</sup>

*Scytalidium hyalinum* is usually isolated from skin and nail infections,<sup>171-173</sup> especially in individuals from the Caribbean and West Africa.<sup>174</sup> Six percent of coal miners in Nigeria were reported to have skin infection solely by this organism.<sup>175</sup> In one immunocompromised patient, this organism was reported to cause a subcutaneous infection with multiple

cyst formation.<sup>176</sup> Two cases of tinea pedis responded to treatment with itraconazole.<sup>177</sup> *Scytalidium* spp. have also been reported to cause keratitis<sup>178</sup> and sinusitis in a lung transplant recipient.<sup>179</sup>

*Trichoderma viride* was reported to cause peritonitis in patients undergoing continuous peritoneal dialysis and invasive infections in immunocompromised patients, including neutropenic cancer patients and transplant recipients.<sup>180-185</sup>

## Treatment

Factors that influence the management of these emerging opportunists include the lack of standardized susceptibility testing, the limited correlation between in vitro antifungal susceptibility testing results and clinical outcome, the difficulty in making an early diagnosis, and the relative resistance to antifungal agents, especially in the setting of severe immunosuppression.

In the normal host, surgery, local instillation of antifungal agents (such as intraarticular, intraocular, other),<sup>186-187</sup> and systemic antifungal therapy may be curative.<sup>117,188,189</sup> In the immunocompromised host, the critical factor for a favorable outcome is recovery from immunosuppression.<sup>7</sup> In these patients, surgery is rarely an option because of severe thrombocytopenia.<sup>118,190,191</sup> Thus, every effort should be made to prevent these infections in this patient population, and to enhance the status of the patient's immune system when infection sets in, including, most importantly, tapering or discontinuation of immunosuppressive drugs. Treatment with granulocyte or granulocyte-macrophage colony stimulating factors (G-/GM-CSF) and CSF-stimulated white blood cells transfusions may also be considered.<sup>10,48,55,189,192,193</sup> A summary of the strategies suggested to reverse immunosuppression is presented in Table 13-2.

Antifungal therapy should be based on the known pattern of susceptibility of the offending pathogen (Table 13-3),<sup>10,189,194-197</sup> and should be continued until resolution of all clinical and laboratory findings of infection and recovery from immunosuppression.<sup>38,57,68,73</sup> Successful therapy



for fungal infections, especially moulds, may require a coordinated medical and surgical approach (Table 13-4).

## Treatment of specific infections

### *Fusarium* spp

In general, localized infection is likely to benefit from surgical debridement, while disseminated infection requires the use of systemic agents and immunotherapy, when possible. Keratitis is usually treated with topical antifungal agents, and natamycin is the drug of choice.<sup>20</sup> More recently, successful treatment with topical and oral voriconazole has been reported.<sup>216</sup> Localized skin lesions in immunocompromised patients deserve special attention. Since the skin may be the source for disseminated and frequently life-threatening fusarial infections, local debridement should be performed and topical antifungal agents (natamycin, amphotericin B) should be used, prior to commencing immunosuppressive therapies.

Because of lack of clinical trials and the critical role of immune reconstitution in the outcome of fusariosis, the optimal treatment strategy for patients with severe fusarial infection remains unclear. The typical antifungal susceptibility profile of *Fusarium* spp. is that of relative resistance to most antifungal agents. However, *F. solani* and *F. verticillioides* are usually resistant to azoles and exhibit higher MICs for amphotericin B than other *Fusarium* spp. By contrast, *F. oxysporum* and *F. moniliforme* may be susceptible to voriconazole and posaconazole.<sup>198-206</sup> High-dose amphotericin B, lipid-based amphotericin B formulations, and combinations of other antifungal agents with amphotericin B have been reported. The response rate to a lipid formulation of amphotericin B appeared superior to that of deoxycholate amphotericin B.<sup>7</sup> Voriconazole and posaconazole have been used as salvage therapy, with acceptable response rates.<sup>207,208</sup>

Data on combination therapy for fusariosis are limited to a few case reports: caspofungin plus amphotericin B,<sup>228</sup> amphotericin B plus voriconazole.<sup>210,211</sup> amphotericin B and terbinafine,<sup>212</sup> and voriconazole plus terbinafine.<sup>232</sup> Given the scarcity of data and the potential publication bias, no solid recommendations can be provided.

In addition to antifungal treatment, the optimal management of patients with fusariosis includes surgical debulking of infected tissues<sup>214</sup> and removal of venous catheters in the occasional patient with confirmed catheter-related fusariosis.<sup>215</sup> The role of G-CSF or GM-CSF stimulated granulocyte transfusions and interferon- $\gamma$  in the adjuvant treatment of fusariosis is not established. However, given the poor prognosis of fusariosis, especially in persistently neutropenic patients, G-CSF and granulocyte transfusions are frequently used. In support, there are isolated case reports of the successful treatment of invasive fusariosis with a combination of medical treatment and some of these measures.<sup>216</sup>

### *Penicillium marneffe*

Amphotericin B has been used for the treatment of severe forms of systemic infection due to *P. marneffe*,<sup>76,217</sup> whereas itraconazole is the preferred drug for treating moderately severe penicilliosis, and for long-term maintenance treatment after a course of amphotericin B.<sup>194,218,219</sup> More recently, voriconazole (oral, or intravenous followed by oral) has been shown to be highly effective for the treatment of penicilliosis.<sup>220</sup>

**Table 13-2** Reversal of Immunosuppression

- Discontinuation or dosage reduction of immunosuppressive drugs (such as corticosteroids, other)
- Infusion of autologous stem cells if delayed marrow engraftment
- Granulocyte transfusion (from donors treated with G-CSF or GM-CSF and dexametasone)
- Administration of recombinant cytokines, particularly interferon- $\gamma$  GM-CSF

G-CSF: granulocyte colony stimulating factor; GM-CSF: granulocyte macrophage colony stimulating factor.

### *Scedosporium* spp

In vitro and in vivo data show that *S. apiospermum* is resistant to amphotericin B and flucytosine and susceptible to itraconazole, voriconazole and posaconazole. By contrast, *S. prolificans* is resistant to both amphotericin B and the azoles.<sup>218</sup>

Optimum management of infection due to *S. apiospermum* includes microbiologic documentation (since these organisms are histologically similar to *Aspergillus* species), and voriconazole or itraconazole.<sup>85,94,96,117</sup> Surgical resection remains the key to a successful outcome if the lesions are localized (e.g., cavitating lung lesion, sinusitis, arthritis or osteomyelitis). The therapeutic outcome is usually poor in the setting of persistent immunosuppression. A combination of interferon- $\gamma$  and antifungal therapy in a patient with granulomatous disease helped control disseminated infection.<sup>221</sup>

The outcome of *S. prolificans* infection is very poor, since no drug appears to be effective.<sup>85</sup> Surgical debridement of infected tissue and recovery of immunosuppression appear to be the major means of halting progression of the infection.<sup>102,208</sup> Anecdotal reports of successful treatment with voriconazole plus terbinafine have been published.<sup>88,213</sup>

### *Paecilomyces* spp.

Both *P. bilacinus* and *P. variotic* are susceptible to the mould-active triazoles (itraconazole, voriconazole and posaconazole) while Amphotericin B is only effective against *P. variotic*. Amphotericin B is the drug of choice for treating *P. variotic* infections, while the mould-active triazoles should be applied for treating infections caused by *P. bilacinus*,<sup>122,127</sup> breakthrough disseminated infection with *P. variotic* developed in a neutropenic patient with leukemia while receiving voriconazole.<sup>131</sup> Surgical intervention should be considered as clinically appropriate.

### *Acremonium* spp

In vitro, *Acremonium* spp are susceptible to amphotericin B and the azoles, including itraconazole, voriconazole and posaconazole.<sup>1,222,223</sup> Clinical data on treatment of infections by *Acremonium* spp. are limited to case reports. Successful clinical outcomes have been observed after treatment with amphotericin B, voriconazole and posaconazole.<sup>224-227</sup> Surgery and catheter removal have also been reported as part of the successful management of these infections.<sup>227-229</sup>

**Table 13-3** In vitro antifungal susceptibility and drug of choice for selected hyalohyphomycosis.

Pathogen	AMB	Flucytosine	Echinocandins	Fluconazole	Itraconazole	Voriconazole	Posaconazole
<i>Fusarium</i> spp.	V	R	R	R	V	V	V
<i>Penicillium marneffei</i>	S*	I-S	NT	I	S**	S**	S
<i>S. apiospermum</i>	I	R	S	I-S	S*	S*	S
<i>Paecilomyces</i> spp.	V*	I	NT	R	S*	S*	S*
<i>Acremonium</i> spp.	S*	R	NT	R	S**	S	S
<i>Scopulariopsis</i> spp.#	I*	R	NT	R	R	R	R

AMB, amphotericin B and its lipid formulations.  
S, susceptible; I, intermediate; R, resistant; NT, not tested; V, variable species-dependent susceptibility.  
*Fusarium solani* and *F. verticillioides* are resistant to azoles, and exhibit higher MICs for amphotericin B than other *Fusarium* spp. *F. oxysporum* and *F. moniliforme* may be susceptible to voriconazole and posaconazole. *P. variotii* is most susceptible to AMB while *P. lilacinus* is best treated with the mould-active azoles.

\*Drug of choice in severe infection.  
\*\*Drug of choice in moderately severe infection, as an alternative agent or as a follow-up to 2 weeks of IV amphotericin B at the dose of 1 mg/kg/day. Secondary prophylaxis with itraconazole (200 mg/day) is recommended in patients with persistent immunosuppression.  
Topical natamycin useful for fusarial keratitis.  
#Terbinafine may be useful for superficial infection  
Modified from Yu VL, Merigan TC, Barriere SL (eds) Antimicrobial Therapy and Vaccine, 1999, p.1105.

**Table 13-4** Indications for Surgical Debridement of Infected Tissue

- Hemoptysis from a single cavitory lung lesion
- Progressive cavitory lung lesion (unless multiple lesions are seen by CT scan)
- Infiltration into the pericardium, great vessels or bronchi, bone or thoracic soft tissue despite antifungal treatment
- Progressive and invasive sinusitis
- Joint/bone infection
- Endophthalmitis
- Skin or nail infection prior to cytotoxic chemotherapy

CT, computed tomography chest scan.

### *Scopulariopsis* spp

*Scopulariopsis* spp. are usually resistant in vitro to antifungal agents including itraconazole, fluconazole, and flucytosine and somewhat susceptible to amphotericin B, miconazole and ketoconazole.<sup>230</sup>

Oral itraconazole and terbinafine and topical natamycin were reportedly effective in treating onychomycosis by this organism.<sup>138,139</sup> Invasive infections may require surgical and medical treatment and are frequently fatal.<sup>145,146,231</sup>

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# Dematiaceous fungi

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The dematiaceous or phaeoid moulds<sup>1</sup> are a heterogeneous group of organisms which include hyphomycetes, coelomycetes, and ascomycetes. Given the large volume of dark etiologic agents, only selected genera/species are included here, as exhaustive descriptions/references are beyond the scope of this work.

These fungi share dark pigmentation resulting from the presence of dihydroxynaphthalene melanin in the cell walls. When invading tissue, dark hyphae may be difficult to visualize with the hematoxylin-eosin stain, but the melanin-specific Masson-Fontana stain facilitates recognition.<sup>2</sup> In culture, melanin imparts colonial pigmentation ranging from buff to pale brown in some species, but predominantly olivaceous to brown to black. While a few of these black moulds may display a mucoid or yeast-like phase, at least initially, most appear filamentous in culture. The number of black moulds reported as etiologic agents continues to escalate with the growing population of immune compromised individuals.

When recovered from patient samples, dematiaceous fungi must be evaluated for their clinical significance based upon compatible histopathology as they are common, ubiquitous organisms known to colonize host sites. The correct identification of the myriad etiologic agents by the laboratory is critical for appropriate patient management. We have therefore arranged this chapter in two sections corresponding to these aims. The first section will provide an outline approach to the identification of selected dematiaceous fungi (Table 14-1), followed by a brief description of the salient features for each genus or species including (1) colony growth characteristics and macroscopic morphology, (2) microscopic morphology, and/or (3) biochemical tests. Detailed descriptions of etiologic agents may be found in other recent works.<sup>3-6</sup> Organisms have been grouped on the basis of their anamorphic (asexual = mitosporic) or teleomorphic (sexual = meiosporic) reproductive propagules, although these groupings do not necessarily reflect phylogenetic relationships.

Categories of black moulds include the hyphomycetes (that bear their conidia free), the coelomycetes (that bear their conidia within enclosed or semi-enclosed conidiomata, such as pycnidia or acervuli, respectively), and the ascomycetes (that bear their ascoconidia within a variety of ascomata, such as cleistothecia, perithecia, gymnothecia and other intermediate

forms). Organisms known to cause only eumycotic mycetoma are considered separately at the end of the first section. The second section contains a discussion of the epidemiology, clinical presentation, diagnosis, and treatment issues for each of the clinical syndromes caused by dematiaceous fungi: chromoblastomycosis, eumycotic mycetoma, and the various manifestations of phaeohyphomycosis.

## A practical approach to identification

### Taxonomy and nomenclature

The proper classification, taxonomy, and nomenclature of dematiaceous fungi, although seldom taught and generally misunderstood, are essential to meaningful communication between taxonomists, mycologists, general microbiologists, and the clinician. While authorities are sometimes in disagreement over taxonomic issues, the frequently changing mycologic nomenclature results from new insights into methods of conidiogenesis, phylogenetic relationships, new species, etc., and more precisely delineates etiologic agents. Also contributing to the taxonomic confusion is the pleomorphic nature of some of the black moulds, particularly those displaying different synanamorphs (different asexual forms of the same fungus), such as *Fonsecaea* species with both *Rhinochrysiella*- and *Cladosporium*-like conidiation, and those in which both the anamorphic and teleomorphic forms are produced in culture, such as dark *Scopulariopsis* species with *Microascus* teleomorphs. The use of multiple taxa for the same organism is also due to retention of obsolete names which should be discouraged.

### Media considerations

The choice of media used for the identification of black moulds is critical, as most of these organisms are either plant pathogens or degrade plant material, and prefer plant-based substrates. The more commonly used formulations include potato dextrose agar (PDA) or the variation, potato flakes agar (PFA), oatmeal agar OA, and malt agar (MA). Color descriptions for organisms included in this chapter are based on those obtained on PFA.



**Table 14-1** Salient features of selected, clinically significant dematiaceous fungi

<b>HYPHOMYCETES (bear their conidia free)</b>	
<b>Colonies may be yeast-like initially or at maturity</b>	
<i>Exophiala dermatitidis</i> ( <i>Wangiella dermatitidis</i> )	Colonies very mucoid; nitrate negative; growth at 40°C; black yeast <i>E. exophialae</i> synanamorph present; most common <i>Exophiala</i> clinical species
<i>Exophiala</i> spp.	Colonies mucoid initially; conidiogenous cells predominantly annellidic; phialides sometimes present; annellated black yeast synanamorph <i>Phaeoannellomyces</i> often seen; many species very similar microscopically; nitrate positive; DNA sequencing facilitates species identification; maximum temperatures vary; frequently seen clinical species include <i>E. xenobiotica</i> , <i>E. oligosperma</i> , <i>E. lecanii-corni</i> and <i>E. phaeomuriformis</i>
<i>Hortaea werneckii</i>	Colonies restricted; broad hyphae, wide annellated zones produce pale brown 1- to multicelled annelloconidia
<b>DNA sequencing necessary for reliable differentiation of <i>A. pullulans</i> and <i>H. dematioides</i></b>	
<i>Aureobasidium pullulans</i>	Cream to pink mucoid colonies initially; later brown to black; hyaline blastoconidia borne synchronously from hyaline hyphae; dark thick-walled chlamydoconidia
<i>Hormonema dematioides</i>	Colonies as above; hyaline blastoconidia produced asynchronously by percurrent proliferation from hyaline and dark hyphae
<b>Colonies are filamentous (woolly, velvety, etc.)</b>	
<i>Alternaria</i> spp.	Large, dark muriform conidia in chains; frequently slow to produce conidia
<b>Large, dark muriform conidia formed singly</b>	
<i>Stemphylium</i> spp.	Conidia produced through nodes on conidiophores (percurrent proliferation)
<i>Pithomyces</i> spp.	Conidia produced on short, straight conidiophores; lacks percurrent proliferation and geniculate conidiophores
<i>Epicoccum</i> spp.	Colonies orange-brown; conidia slow to form in sporodochial areas
<i>Ulocladium</i> spp.	Conidia formed on geniculate conidiophores
<b>Large, dark conidia formed singly with transverse septa</b>	
<i>Bipolaris spicifera</i>	Bipolar germination, geniculate conidiophores; 3 distosepta, 4 cells; flattened hilum
<i>Bipolaris hawaiiensis</i>	Bipolar germination, geniculate conidiophores; predominantly 5 distosepta, 6 cells; flattened hilum
<i>Curvularia</i> spp.	Geniculate conidiophores; middle cell swollen, producing curvature in conidia
<i>Exserohilum rostratum</i>	Geniculate conidiophores; 7–9 distosepta; 8–10 cells; prominent dark basal and distal septa; truncate, protruding hilum
<i>Fonsecaea</i> spp.	Small conidia with multiple types of conidiation. Conidia form from swollen denticles which give rise to secondary and tertiary conidia; also formed on sympodial conidiophores like <i>Rhinochadiella</i> , in chains as in <i>Cladosporium</i> , and occasionally from phialides, as in <i>Phialophora</i>
<b>Small conidia on geniculate conidiophores (sympodial development)</b>	
<i>Fonsecaea</i> spp.	Described above
<i>Rhinochadiella</i> spp.	Long, erect, unbranched sympodial conidiophores; 1-celled pale ellipsoidal conidia (3–7.5 × 1.7–2.5 µm) borne on crowded denticles; an <i>Exophiala</i> yeast synanamorph may be present

**Table 14-1** Salient features of selected, clinically significant dematiaceous fungi—cont'd

<i>Ramichloridium mackenzie</i>	Relatively few conidia per fertile part of geniculate conidiophore; 1-celled conidia pale brown, ellipsoidal (5–10 × 3–6 µm) with a prominent truncate hilum; poor growth at 25°C; prefers 35°C and above
<i>Veronaea botryosa</i>	Long, brown conidiophores; pale brown, 2-celled conidia with a rounded apex and truncate base borne from closely spaced intercalary conidiogenous cells
<b>Small conidia forming in chains</b>	
<i>Cladosporium</i> spp.	Conidiophores simple or branched, with or without nodes or swellings; ramoconidia ("shield cells") give rise to branching chains of fragile, dark, mostly 1- or 2-celled conidia with prominent attachment scars (hila)
<i>Cladophialophora</i> spp.	Similar to <i>Cladosporium</i> spp. but lack conidiophores, "shield cells", and prominent hila; conidia are non-fragile (remain intact in chains)
<i>Acrophialophora fusispora</i>	Colonies centrally dark front and reverse; unbranched, erect, brown, echinulate conidiophores are anchored by a foot cell; chains of conidia with fine or coarse spirals produced from apex of brown conidiophores and inflated phialides on hyaline hyphae; growth at 40°C
<i>Scopulariopsis</i> spp.	Conidiogenous cells annellidic; several dark species are anamorphs of <i>Microascus</i> spp.
<b>Small conidia mostly produced singly by phialides/adelophiades</b>	
<i>Phialophora</i> spp.	Phialides may have prominent collarettes as in <i>P. verrucosa</i> and <i>P. americana</i>
<i>Pleurostomophora</i> spp.	Colonies are brown and phialides are slender rather than flask-shaped. <i>P. richardstae</i> produces cylindric conidia from phialides with inconspicuous collarettes, and globose conidia from phialides with flaring collarettes.
<i>Phaeoacremonium</i> spp.	Colony colors range from buff to pale yellow, pale to dark pink to various shades of brown; hyphae brown; conidiophores often have small warts (exudate); three distinct types of phialides, i.e., types I, II, and III; polyphialides may be present; 1-celled conidia aggregate at apices of phialides and are commonly reniform (kidney-shaped) to allantoid (sausage-shaped)
<i>Phialemonium</i> spp.	Colonies buff to gray to yellow; conidiogenous cells phialides and adelophialides; <i>P. obovatum</i> has obovate conidia and a green diffusing pigment; <i>P. curvatum</i> may also form conidia in sporodochia
<i>Lecythophora</i> spp.	Colonies moist, salmon to orange; conidiogenous cells primarily adelophialides; conidia aggregate at apices of conidiogenous cells; <i>L. mutabilis</i> distinguished from <i>L. hoffmannii</i> by dark chlamydoconidia
<b>Small conidia formed on denticles or ampulliform swellings</b>	
<i>Ochroconis gallopavum</i>	Colonies are brownish with a red diffusing pigment; 2-celled, clavate conidia borne from denticles; growth at 45°C; no growth on media containing cycloheximide
<i>Myceliophthora thermophila</i>	Colonies light brown, powdery, ill-defined margin; conidia borne from ampulliform swellings are hyaline and smooth initially becoming rough and brown at maturity; growth at 48°C
<i>Scytalidium dimidiatum</i>	Two-celled arthroconidia. Rapidly growing woolly colonies filling plate within a few days; 1- and 2-celled, dark or hyaline arthroconidia not separated by disjunct cells; thin hyaline and wide (10–12 µm) dark or hyaline hyphae; coelomycetous synanamorph, <i>Nattrassia mangiferae</i> , requires several weeks to mature
<i>Madurella</i> spp.	Isolates usually sterile (fail to produce conidia). Colonies very slow growing and often heaped; dark brown to black; diffusible brown pigment; unlike <i>M. grisea</i> , <i>M. mycetomatis</i> grows at 40°C and fails to assimilate sucrose; precise identification facilitated by ITS sequencing

(Continued)

**Table 14-1** Salient features of selected, clinically significant dematiaceous fungi—cont'd

<b>COELOMYCETES (bear their conidia within enclosed/semi-enclosed structures; organisms treated here have pycnidial conidiomata)</b>	
<i>Natrassia mangiferae</i>	Coelomycetous conidiogenous cells phialidic. Conidia form after extended incubation and are versicolored (middle cell darker); 2-celled, dark arthroconidia formed by the <i>Scytalidium dimidiatum</i> synanamorph
<i>Phoma</i> spp.	Conidia small, aseptate, hyaline, often guttulate; <i>Pleurophoma</i> , <i>Pleurophomopsis</i> , and <i>Pyrenochaeta</i> spp. are similar; consult Boerema et al. <sup>90</sup> for differentiation
<i>Coniothyrium fuckelii</i>	Similar to <i>Phoma</i> but with pale brown conidia; most authorities would place it in the genus <i>Microsphaeropsis</i> ; <i>Paraconiothyrium</i> and <i>Microsphaeropsis</i> are similar species; precise identification facilitated by ITS sequencing
<i>Lasiodiplodia theobromae</i>	Large, variably shaped, ostiolate pycnidia, sometimes with setae; conidiogenous cells annellidic; large conidia 20–30 × 10–15 μm, initially aseptate and hyaline; 1 septate, dark, longitudinally striate at maturity
<b>ASCOMYCETES (produce ascomata, asci, and ascospores in culture)</b>	
<b><i>Chaetomium</i> spp. form large, reddish-brown, elliptical ascospores within perithecia covered with setae (hairs)</b>	
<i>Chaetomium globosum</i>	Setae coiled; ascospores subglobose; growth at 35°C; no growth at 42°C
<i>Chaetomium atrobrunneum</i>	Colonies gray, setae mostly straight; ascospores narrowly fusoid; growth at 42°C
<i>Chaetomium perlucidum</i>	Very similar to <i>C. atrobrunneum</i> in colony morphology, setae, and ascospores size; growth at 42°C
<i>Chaetomium strumarium</i>	Colonies pale with red diffusing pigment; also known as <i>Achaetomium strumarium</i> ; growth at 42°C
<i>Leptosphaeria</i> spp.	Colonies dark, slow-growing; asci contained within non-ostiolate ascomata; ascospores hyaline, mostly with 4–6 septa; <i>L. senegalensis</i> and <i>L. thompkinsii</i> distinguished by ascospores features
<b><i>Microascus</i> spp. treated have very similar, dark <i>Scopulariopsis</i> anamorphs and reddish-brown ascoconidia</b>	
<i>Microascus cinereus</i>	Short perithecial necks; orange segment-shaped ascospores
<i>Microascus cirrosus</i>	Longer perithecial necks; heart-shaped ascospores
<i>Microascus trigonosporus</i>	Longer perithecial necks; triangular ascospores
<i>Pseudallescheria boydii</i>	Ascoma cleistothecial. Discussed under agents of hyalohyphomycosis in this text

Laboratories are encouraged to be consistent in the medium used so as to become familiar with variations in color with different genera/species. Other media useful for the production of conidiomata/ascomata for the phaeoid coelomycetes/ascomycetes, respectively, include V-8 agar, carnation leaf agar (CLA), and various other plant substrates. Sabouraud dextrose agar, frequently employed in the battery of primary media, should not be used for black mould identification. Colonies fail to develop their characteristic color on this medium and conidia/sporulation is generally delayed, poor or absent.

## Growth rate and macroscopic morphology

The growth rate of dematiaceous fungi is often described as slow, moderate or rapid, but these terms are somewhat ill defined. A more precise method of documenting growth

rate is measurement of the colony's radius or diameter after a specified number of days on a given medium and at a given temperature. Such a scheme facilitates species identification of *Phaeoacremonium* species utilizing an 8-day incubation period on malt agar at 25°C.<sup>7</sup> The colonial morphology of black moulds varies from those that are initially mucoid or yeast-like and remain so at maturity, to those that may have a mucoid phase but eventually become filamentous, to those that always appear filamentous. Morphology and color are significantly influenced by environmental conditions, thus the importance of a standardized medium when describing gross features. On PFA, color descriptions for the black moulds range from buff to pale brown to jet black. Reverse coloration may also be significant, as in *Acrophialophora fusispora* displaying a tan to gray front with a centrally dark reverse.<sup>8</sup> The use of a dissecting microscope with back lighting is also



extremely helpful for viewing and picking out structures of interest that may be on or below the surface of the medium. Such structures may include sporodochia (aggregated conidiophores and conidia) and various types of conidiomata or ascomata that can be further examined microscopically in a tease mount.

## Microscopic morphology

Some commonly used techniques for demonstration of microscopic features include a tease mount, a tape mount, and/or a slide culture. Tease preparations are often used to pick out selected areas from a plate or are useful when isolates are grown in tubes or bottles with agar slants and limited access. Temporary tape mounts may provide a wealth of information as methods of conidiogenesis, conidiophores, conidia, etc. are easily observed for many of the dark moulds. Slide cultures prepared on media known to promote conidiation/sporulation are preferred, however, as they provide permanent slides for review and comparison with other isolates, and preserve the nature of delicate structures. Various mounting fluids may be used with these preparations, the most common being lactophenol cotton blue.

Recognition of the various microscopic structures produced by hyphomycetes is essential for identification. These features have been described in detail elsewhere<sup>3,4</sup> and will only be briefly reviewed here. Conidiation for most of the dark mitosporic fungi is primarily of two types: blastic or thallic. Blastic conidia (blastoconidia) are by far the most prevalent and are produced by a “blowing out” process through pores or various specialized conidiogenous cells such as annellides (possessing rings or annellations of cell wall material), phialides (with cell wall extensions at their apices called collarettes), and adelophialides (reduced phialides lacking a basal septum). These conidiogenous cells may be supported by conidiophores, and in some genera, such as *Bipolaris*, *Curvularia* and others, may have a sympodial growth pattern resulting in a “zig-zag” appearance. Thallic conidia, termed arthroconidia, are not “blown out” but formed from preexisting hyphae with subsequent release by various mechanisms. Dark arthroconidia are seen in the genera *Scytalidium*, *Oidiodendron*, and others. Conidia are differentiated by their number, size, orientation in relation to each other, and patterns of septation. Blastoconidia may accumulate at the apices of the conidiogenous cells, such as in *Exophiala* spp., or form in long or short chains, as in *Cladophialophora* and *Cladosporium*, respectively. Distinctive darkened points of attachment, known as hila, may be present in some species. In genera producing large conidia, such as *Bipolaris*, *Curvularia*, *Exserohilum*, and *Alternaria*, the numbers and types of septations, whether chains are formed or not and orientation of germ tubes in relation to the long axis of the germinating conidia are all important characteristics.

In the coelomycetes conidiogenous cells line the cavities of enclosed or semi-enclosed structures such as pycnidia or acervuli, respectively, and may be annellidic or phialidic; however, their differentiation is often problematic. Conidial size, shape, color, and septations are important features for identification.

Phaeoid ascomycetes are identified based upon the size, shape, and morphology of their ascomata, asci, and ascospores. Ascomata are commonly of three types: cleistothecia

(completely enclosed); perithecia (with an opening through which ascospores are released); and gymnothecia (consisting of a loose arrangement of hyphae surrounding the ascospores). Intermediate forms of conidiomata and ascomata may also be present.

## Temperature tolerance

Determining an organism's temperature tolerance may facilitate genus and/or species identification. Growth at 35°C has also been used to suggest pathogenicity, although case reports provide evidence of systemic disease with strains that fail to grow at this temperature in culture.<sup>9</sup> Black moulds with the ability to grow at 40°C or above such as *Exophiala* (*Wangiella*) *dermatitidis*, *Cladophialophora bantiana*, *Ochroconis gallopavum*, *Ramichloridium mackenziei*, *Myceliophthora thermophila*, *Acrophialophora fusispora*, and *Microascus cinereus* are often neurotropic and agents of central nervous system phaeohyphomycosis.<sup>10</sup>

## Biochemical and serologic tests

Several biochemical tests have been advocated to aid in the identification of black moulds, but most have limited applications. The nitrate assimilation test is generally accepted as a useful means of differentiating *Exophiala* (*Wangiella*) *dermatitidis* (negative) from other *Exophiala* species (positive). Carbohydrate assimilation tests and other tests of “nutritional physiology” are useful in selected situations, as is the ability of organisms to grow on media containing 0.05% cycloheximide (as found in Mycosel (BBL) or Mycobiotic (Difco) agars). Salt tolerance testing utilizing varying concentrations of sodium chloride from 3% to 15% may also facilitate the identification of certain halophilic species. Proteolytic activity has been used in the past as a tool for differentiation, but because of poor reproducibility this technique is not currently recommended.<sup>11</sup> The exoantigen test<sup>12</sup> has been developed in individual research laboratories for many dematiaceous species,<sup>13</sup> but because of cross-reactions, it does not allow identification to the species level, and the necessary reagents are not commercially available. Other immunologic methods have shown promise in some research settings, but these have not been found to be practical for widespread use.

## Molecular techniques

Modern molecular methods are being used extensively to redefine important phylogenetic relationships among the dematiaceous fungi. Polymerase chain reaction (PCR) ribotyping, random primed PCR with DNA hybridization, and comparisons of ribosomal or internal transcribed spacer (ITS) region DNA sequences are among the techniques that have been used. The most frequently used method compares highly conserved ribosomal or the nearby ITS region sequences as the basis for determining relatedness. This work has confirmed some previously suspected relationships, has revealed others that were unexpected on the basis of morphologic criteria, and is proving to be extremely useful in delineating organisms within defined species and species complexes.

Molecular identifications provided by research/reference laboratories should always be correlated with morphologic features of the isolates to insure that contaminating organisms have not been inadvertently sequenced. Several molecular techniques are also being investigated for the detection and/or identification of fungi from direct clinical material – a method which, when perfected, will significantly impact clinical mycology laboratories. Despite these advances, most clinical isolates will continue to be identified using classic morphologic features and selected physiologic/biochemical tests.

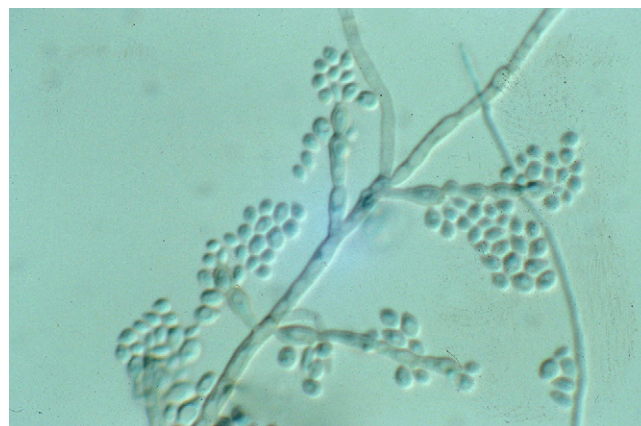
## Descriptions of etiologic agents

### Hyphomycetes

Colonies may be yeast-like initially or at maturity

The “black yeasts” are anamorphic, hyphomycetous fungi that may form darkly pigmented budding cells at some point in their development, and these have been shown by molecular studies to be members of two distantly related groups.<sup>14</sup> *Exophiala* species and related organisms are anamorphs of members of the ascomycete family Herpotrichiellaceae, whereas the teleomorphs associated with *Aureobasidium* and *Hormonema* species, and *Hortaea werneckii* are in the family Dothideaceae.<sup>15</sup> The pleomorphic nature of *Exophiala* species, their methods of conidiogenesis (annellidic vs phialidic, or both), their extremely similar microscopic features, and their lack of definitive biochemical tests, for most species, have made identification and differentiation notoriously difficult. Notable exceptions include the large conidiophores in *E. spinifera* and *E. attenuata*, and the thermotolerance and absence of nitrite assimilation in *E. dermatitidis*. Molecular characterization of *Exophiala* species, particularly sequences of the small-subunit (SSU) and the ITS domains of ribosomal DNA, has facilitated differentiation and a redescription of the heterogeneous species, *E. jeanselmei*, and has identified several new species such as *E. oligosperma*<sup>16</sup> and *E. xenobiotica*.<sup>17</sup>

Utilizing these tools, Zeng *et al*<sup>18</sup> recently reidentified 187 clinical isolates of *Exophiala* from the USA by sequence analysis. Most had previously been identified as *E. jeanselmei* or other *Exophiala* species. Their aim was to correlate morphologic with molecular identification, determine the spectrum of clinically relevant species, and ascertain any significant differences in *in vitro* susceptibility patterns. As expected, many taxa were incorrectly identified using morphologic/physiologic criteria alone. Of the 23 known species of *Exophiala*, 17 cause disease or colonization in humans and animals. Thirteen of the 17 potentially pathogenic species were identified in this study, with *E. dermatitidis*, *E. oligosperma*, and *E. xenobiotica* accounting for approximately 70% of all strains. *Exophiala phaeomuriformis* was the next most common species. *Exophiala jeanselmei*, regarded in the literature as the major agent of human disease, was seldom seen (3.8%). Of note, on the basis of morphologic and physiologic features alone, *E. dermatitidis* was correctly identified 97% of the time, while the remaining species were mostly identified as *E. jeanselmei*. Excluding *E. dermatitidis*, molecular characterization appears necessary for definitive species identification.

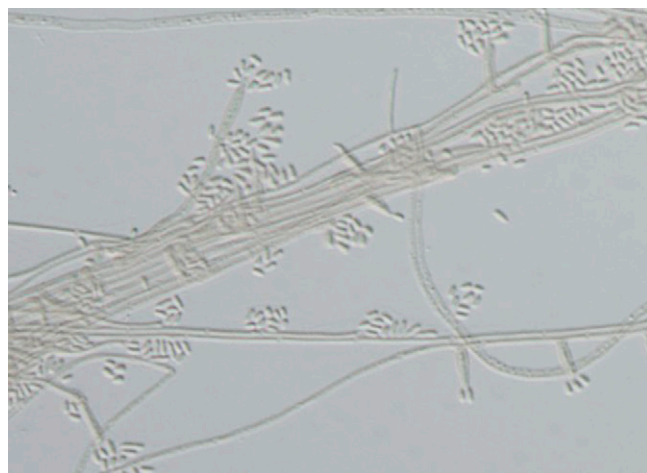


**Figure 14-1** *Exophiala (Wangiella) dermatitidis*. Conidiophores and conidia (×920).

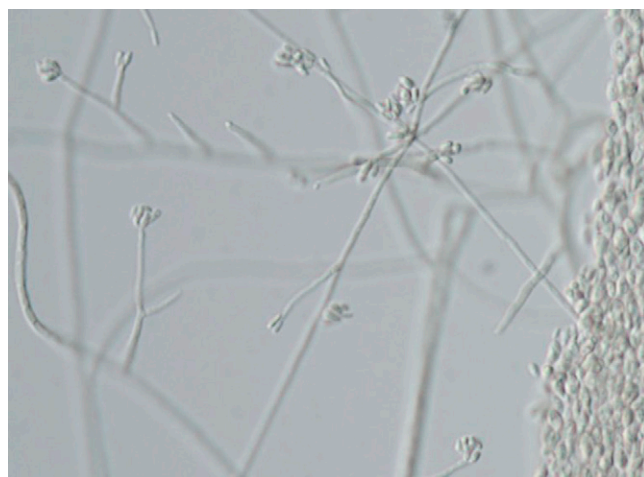
*Exophiala (Wangiella) dermatitidis*. *Exophiala dermatitidis* (*Wangiella dermatitidis*) is an etiologic agent of subcutaneous, ocular, systemic, and cerebral forms of phaeohyphomycosis. The neurotropic nature of the organism is associated with high mortality.<sup>19</sup> Respiratory tract colonization is common in cystic fibrosis (CF) patients in Europe and has been recently reviewed.<sup>20</sup> Strains isolated from cases of invasive disease and from CF patients have been shown to be genetically similar, suggesting that host factors or mode of exposure are important determinants of the form of disease caused by this organism.<sup>21</sup> Growth is initially yeastlike, black, and mucoid, with most colonies eventually becoming velvety and olivaceous gray. The yeastlike component is a prominent feature of this species and represents the budding black yeast *Exophiala exophialae* synanamorph.<sup>16</sup> The filamentous form consists of septate, pigmented hyphae. Single-celled conidia (2–2.5 × 4–6 µm) are subglobose to elliptical and accumulate at the apices of conidiogenous cells (Fig. 14-1).

**Other *Exophiala* species.** *Exophiala oligosperma* (*Melanconolus oligospermus*) (Fig. 14-2) is similar to *E. jeanselmei* and is an agent of olecranon bursitis<sup>22</sup> and central nervous system disease, previously published as *Phialophora dermatitidis*<sup>23</sup> and as an *Exophiala* species.<sup>24</sup> *Exophiala xenobiotica* (Fig. 14-3), also similar to *E. jeanselmei*, has the potential for infection in humans as well as an environmental preference for soils and water containing xenobiotics such as aromatic hydrocarbon pollutants.<sup>17</sup> *Exophiala phaeomuriformis* (*Sarcinomyces phaeomuriformis*) (Fig. 14-4) is similar to *E. dermatitidis* in being very mucoid and growing at 40°C; it differs by being nitrate positive.<sup>25</sup> *Exophiala spinifera* has been reported as a causative agent of both phaeohyphomycosis<sup>26</sup> and chromoblastomycosis.<sup>27</sup> *Exophiala spinifera* differs from most other *Exophiala* spp. by having longer, multicellular, spinelike annellophores that are usually noticeably darker at their bases and terminate in annellides with long tapering tips (Fig. 14-5). It is similar to the newly described *E. attenuata*.<sup>28</sup> *Exophiala* species have been isolated from compounding fluids in hospital pharmacies<sup>29,30</sup> and *Exophiala mesophila* has been recovered from dental water lines being treated to reduce bacterial counts.<sup>31</sup>

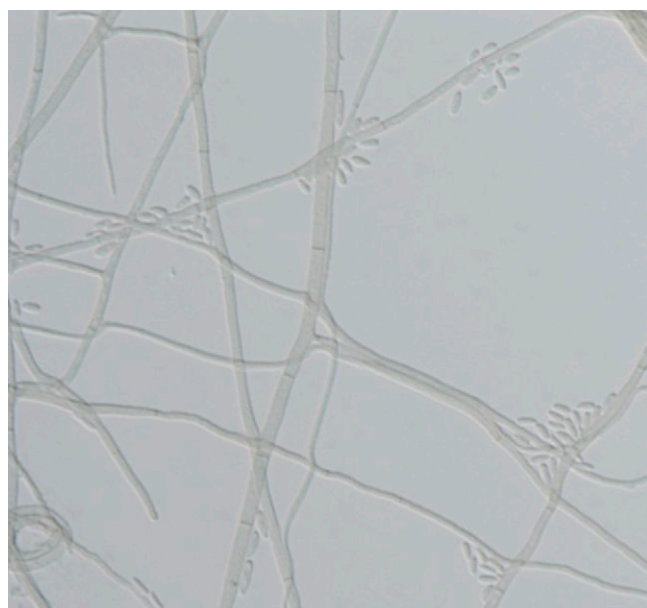
*Hortaea werneckii* (*Phaeoannellomyces werneckii*, *Exophiala werneckii*). *Hortaea werneckii* is the etiologic agent of tinea



**Figure 14-2** *Exophiala oligosperma*. Ellipsoidal annelloconidia born from tapering annellides and from intercalary conidiogenous loci (×920).



**Figure 14-4** *Exophiala phaeomuriformis*. Black yeast synanamorph (right side), conidiogenous cells and ellipsoidal annelloconidia (×920).



**Figure 14-3** *Exophiala xenobiotica*. Ellipsoidal to allantoid (sausage-shaped) annelloconidia born from intercalary conidiogenous loci (×920).



**Figure 14-5** *Exophiala spinifera*. Long septate annellides give rise to narrow ellipsoidal annelloconidia (×920).

*nigra*, a superficial cutaneous mycosis typically involving either the palms of the hands or soles of the feet, and frequently acquired in subtropical coastal locations. Infection with this salt-tolerant organism is postulated to occur through exposure of superficially abraded skin to drying tidal pools.<sup>32</sup> The olivaceous to black colonies are smooth, slimy, and yeast-like and show restricted growth. Hyphae (width up to 6 μm) are densely septate, thick-walled, and brown. Intercalary or lateral conidiogenous cells with prominent annellations produce smooth one- to two-celled ellipsoidal conidia (3.5–4.5 × 7–9.5 μm) that are initially hyaline but later become pale olivaceous. Conidia may exhibit budding and often develop into aggregates of chlamydoconidia. Tolerance of 10% NaCl, lack of growth at 37°C, and the broad annellated zones allow differentiation from *Exophiala* species.

*Aureobasidium pullulans* and *Hormonema dematioides*. *Aureobasidium pullulans* and *Hormonema dematioides* have traditionally been distinguished on the basis of differences in their modes of conidiogenesis: primarily synchronous in *Aureobasidium* and percurrent in *Hormonema*. This characteristic can be examined by use of the Dalmau plate method as described for demonstrating chlamydoconidia in *C. albicans*.<sup>4</sup> Yurlova et al<sup>33</sup> noted, however, that it is often difficult to distinguish between these modes of conidiogenesis, and they suggested differences in the number of conidiogenous loci and physiologic tests as methods for differentiation. These differences, however, are also small and variable, thus requiring DNA sequencing for definitive separation.<sup>34</sup>

Many human infections by *A. pullulans* have followed traumatic inoculation. Published reports have included keratitis, onychomycosis, cutaneous and subcutaneous phaeohyphomycosis, osteomyelitis of the mandible after tooth extraction, systemic phaeohyphomycosis in both HIV-infected and non-infected individuals, and dialysis-associated peritonitis.<sup>35</sup> *Hormonema dematioides* is recognized as an opportunistic pathogen of conifers and possibly other plants. This organism has been reported as a rare cause of cutaneous





**Figure 14-6** *Hormonema dematioides*. Brown thick-walled septate hyphae and smooth ellipsoidal hyaline conidia (×920).



**Figure 14-7** *Alternaria* species. Chain of muriform conidia with apical beaks (×460).

phaeohyphomycosis<sup>36</sup> and fungal peritonitis.<sup>37</sup> The growth rate for both organisms is moderate to rapid. Most strains are initially cream or pink-colored, later changing at least partly to brown or black (often in sectors), but even early growth in some cases is gray or black.<sup>33</sup> Colonies are smooth and moist, often with a slimy exudate. Branched, septate, hyaline hyphae (3–12  $\mu\text{m}$ ) give rise to ellipsoidal blastoconidia (4–7  $\times$  8–16  $\mu\text{m}$ ), which may vary in shape and size and often have indistinct hila. Conidia often bud to produce secondary conidia. Darkly pigmented hyphae form chains of thick-walled one- to two-celled pigmented chlamydoconidia that may also produce blastoconidia (Fig. 14-6). Growth for both organisms is variable at 35°C and neither grows on media containing cycloheximide. Specialized testing of nutritional physiology is discussed in detail elsewhere.<sup>35</sup>

#### Large, dark muriform conidia in chains

***Alternaria* species.** *Alternaria* is a large genus composed mostly of saprobic or plant pathogen species. Infections caused by *Alternaria* species, and cited primarily as *A. alternata*, include cutaneous disease, mycotic keratitis, paranasal sinusitis, complicated in some cases by osteomyelitis, pulmonary nodules, and dialysis-associated peritonitis. *Alternaria* species have also been implicated in the development of cases of asthma and hypersensitivity pneumonitis. Other species cited as etiologic agents include *A. chlamydospora*,<sup>38</sup> *A. dianthicola*, *A. infectoria*, *A. longipes*, and *A. tenuissima*.<sup>39-41</sup> Several of these species are morphologically distinct but indistinguishable from *A. alternata* by ITS sequencing. Growth of *Alternaria* is rapid, with olivaceous to gray to black woolly colonies. Conidiophores are erect, septate, and geniculate. Large brown, muriform conidia with beaks are borne singly or in chains (Fig. 14-7). Identification to the species level is difficult and normally performed in reference laboratories.

#### Large, dark muriform conidia formed singly

***Stemphylium*, *Pithomyces*, *Epicoccum*, *Ulocladium* species.**

*Stemphylium* species have dark conidiophores that are swollen at their tips as a result of percurrent proliferation (growing through the tip of the conidiogenous cell) and give rise to single round or oval muriform conidia that may be constricted at

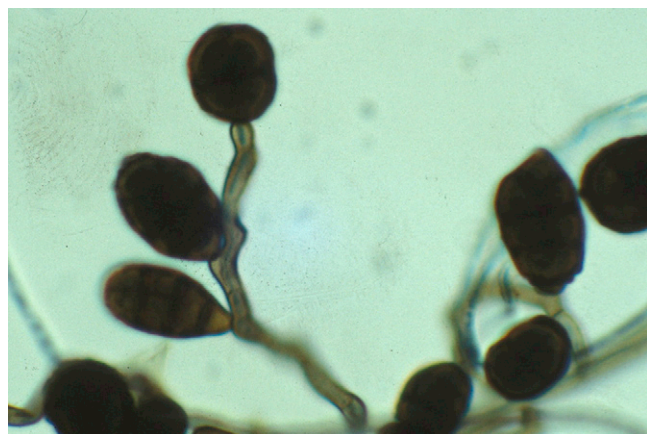


**Figure 14-8** *Pithomyces chartarum*. Short conidiophores and echinulate muriform conidia (×920).

their central septum. The conidiophores of *Pithomyces* species are short, peglike lateral branches from the vegetative hyphae. Conidia are borne singly, are pyriform to elliptical and rough, with either a muriform septal pattern or transverse septa only (Fig. 14-8). *Ulocladium* species produce conidia in a sympodial fashion from geniculate conidiophores; conidia may be smooth or rough (Fig. 14-9). *Ulocladium atrum* has recently been implicated as an agent of keratitis.<sup>42</sup> *Epicoccum* species are recognized by their intense, distinctive yellow to orange-brown color, a brown diffusing pigment, and by conidial production restricted to sporodochia areas rather than throughout the culture.

#### Large, dark conidia formed singly with transverse septa

***Bipolaris* species.** The common species inciting human disease include *B. australiensis*, *B. hawaiiensis*, and *B. spicifera*. Growth is moderately rapid, with mature colony formation generally within a week. Colonies are woolly, gray to black, with a black reverse. Hyphae are septate, and conidiophores are erect, septate, and geniculate as a result of sympodial development. Conidia are straight, rounded at both ends, have a dark, flattened hilum on the basal cell, and one to several distosepta (septa that do not extend to the cell wall with cells



**Figure 14-9** *Ulocladium* species. Geniculate conidiophores with verrucose muriform conidia (×920).



**Figure 14-10** *Bipolaris spicifera*. Geniculate conidiophore and conidia. Conidia have predominantly three distosepta (×920).

enclosed within sacs) and often finely roughened walls; conidial size and number of septations are key features used to differentiate between species. Germination is from both poles, or end cells, hence the genus “*Bipolaris*.”

*Bipolaris spicifera* is the most commonly recovered species. Disease associations include subcutaneous lesions, sinusitis, keratitis, peritoneal dialysis-associated peritonitis, and central nervous system phaeohyphomycosis.<sup>43</sup> Mature conidia (6–13 × 16–39 μm) are oblong to cylindric, with a small hyaline area just above the hilum. There are normally three distosepta, or more rarely two or four (Fig. 14-10). *Bipolaris australiensis* is a relatively rare clinical isolate from cutaneous and subcutaneous lesions. Conidia (6–13 × 14–34 μm) are oblong to ellipsoidal and lack the suprahilar hyaline area described for *B. spicifera*. Three distosepta are frequent, but 10–20% of conidia have four or five septations. This variability is often useful in identifying this species. *Bipolaris hawaiiensis*, often an aggressive species, has been isolated from cases of invasive sinusitis, brain lesions, peritoneal dialysate, sputum samples, and lung tissue. Marijuana use has been noted as a possible risk factor.<sup>44</sup> The ellipsoidal conidia (4–9 × 16–34 μm) are narrower than those of the other species, and differ by typically having four or five distosepta (Fig. 14-11).

*Curvularia* species. *Curvularia* species, common inhabitants of dead plant material, are causative agents of fungal keratitis, sinusitis, onychomycosis, “black grain” mycetoma, subcutaneous phaeohyphomycosis, peritonitis and systemic phaeohyphomycosis, with many infections occurring in apparently immunocompetent hosts.<sup>3</sup> Recent reports also include fatal cerebral phaeohyphomycosis in an immunocompetent host,<sup>45</sup> endophthalmitis,<sup>46</sup> and contamination of saline-filled breast implants.<sup>47</sup> Etiologic agents include *C. geniculata*, *C. lunata*, *C. pallescens*, *C. senegalensis*, *C. brachyspora*, *C. clavata*, *C. verruculosa*, and *C. inaequalis*.<sup>48</sup> Colonies display rapid growth and are olivaceous to grayish (bluish) black. Conidiophores are darkly pigmented and geniculate as a result of sympodial development. Curved conidia, sometimes subtle, result from an enlarged central cell, which is also darker than the other surrounding cells. *Curvularia lunata* is a causative agent of onychomycosis, dialysis-associated peritonitis, eumycotic mycetoma, mycotic keratitis and sinusitis, subcutaneous



**Figure 14-11** *Bipolaris hawaiiensis*. Geniculate conidiophores, conidia with flattened hila and predominantly five distosepta (×460).

phaeohyphomycosis, and systemic phaeohyphomycosis, including disseminated disease.<sup>49</sup> The conidia of *C. lunata*, the most frequently encountered species, typically have three septa and four cells (Fig. 14-12). *Curvularia lunata* var. *aeria* produces large, upright stroma readily visible in culture with the naked eye.

*Exserohilum* species. This genus includes three human pathogens: *E. rostratum*, *E. longirostratum*, and *E. mcginisii*. Growth is rapid and colonies are woolly and gray to black in color. Hyphae are septate and dematiaceous. Conidiophores are geniculate as a result of sympodial development. Conidia may be straight, curved or slightly bent and are distinguished by having prominent, protruding hila. The most frequently isolated species, *E. rostratum*, is the etiologic agent of keratitis, sinusitis, cutaneous and subcutaneous phaeohyphomycosis. The first septum at each end of each conidium is noticeably darker in pigment than the other septa, and the end cells are frequently paler than the other cells. These two conidial features are the most useful distinguishing characteristics for this organism. Conidial size (9–23 × 30–128 μm) is quite variable between strains and even for a single isolate. Conidia can have 4–14 septa, but most strains have 7–9 (Fig. 14-13).





**Figure 14-12** *Curvularia lunata*. Geniculate conidiophore giving rise to four-celled conidia, with the middle cell being inflated ( $\times 920$ ).

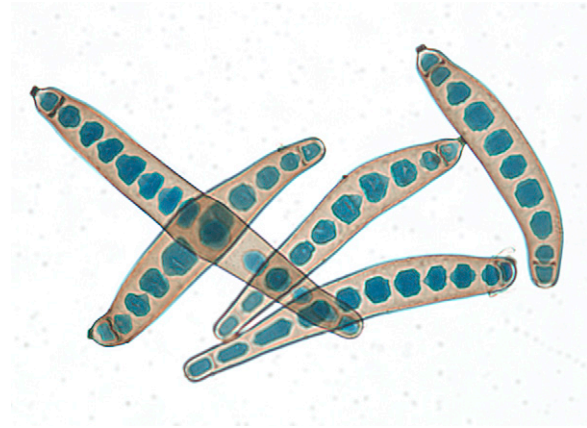
An uncommon clinical isolate, *E. longirostratum*, has been isolated from an infected heart valve prosthesis. Similar to *E. rostratum*, it differs by longer, frequently bent conidia ( $12\text{--}20.5 \times 100\text{--}430\ \mu\text{m}$ ) having 13–21 septa; shorter conidia ( $13\text{--}19 \times 38\text{--}80\ \mu\text{m}$ ) are normally present also. Some consider *E. longirostratum* synonymous with *E. rostratum*.<sup>43</sup> *Exserohilum mcginnsii* is an infrequent agent of fungal sinusitis. The conidia are similar to those of *E. rostratum* ( $10\text{--}15 \times 64\text{--}100\ \mu\text{m}$ ) but differ by lacking dark basal and distal septa, and by possessing irregular “warty” projections on their outer walls.

Small conidia formed by multiple types of conidiation *Fonsecaea* species. *Fonsecaea pedrosoi* and the recently described *F. monophora* are agents of chromoblastomycosis, with *F. pedrosoi* being the most common cause worldwide. *Fonsecaea monophora* (Fig. 14-14), morphologically similar to *F. pedrosoi*, is also neurotropic, being an agent of cerebral phaeohyphomycosis in a recent report by Surash et al,<sup>50</sup> as well as having been previously published as neurotropic under the name *F. pedrosoi*. Molecular characterization facilitates the separation of these agents. Growth is slow for both species, with dark brown to black, velvety colonies maturing in 2–4 weeks.

*Fonsecaea* species are pleomorphic and capable of producing four distinct types of conidiation. The “*Fonsecaea*” type, the most characteristic form, consists of septate, compactly sympodial conidiophores with slightly swollen tips, which give rise to single-celled, ovoid primary conidia ( $1.5\text{--}3 \times 2.5\text{--}6\ \mu\text{m}$ ). The primary conidia can in turn form secondary conidia, which can form tertiary conidia, but longer chains are not formed (see Fig. 14-14). *Fonsecaea compacta* is now considered synonymous with *F. pedrosoi*.<sup>51</sup> Conidial formation can also be of “*Rhinochadiella*,” “*Cladosporium*” or more rarely “*Phialophora*” types, the microscopic morphologies of which are discussed under those organisms elsewhere in this chapter.

Small conidia on geniculate conidiophores (sympodial development)

*Rhinochadiella* species. Potentially pathogenic *Rhinochadiella* species are *R. aquaspersa*, a rare agent of chromoblastomycosis, and *R. atrovirens*, which was reported to have caused cerebral phaeohyphomycosis in a patient with AIDS.<sup>52</sup>



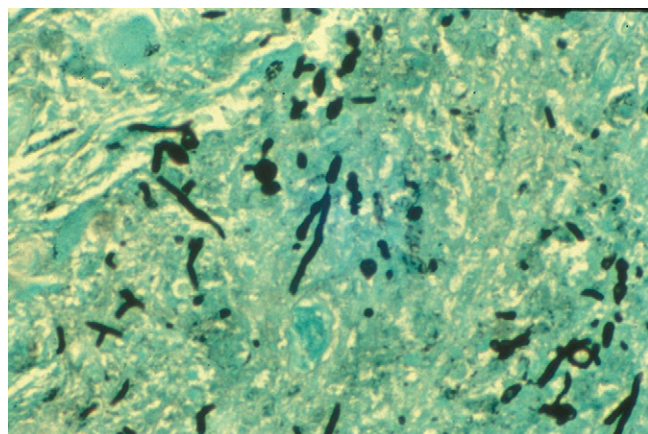
**Figure 14-13** *Exserohilum rostratum*. Conidia displaying several distosepta, prominent dark basal and distal septa, and protruding hila ( $\times 1200$ ).



**Figure 14-14** *Fonsecaea monophora*. Complex fruiting structures with short conidial chains ( $\times 920$ ).

Growth is moderately rapid in *R. atrovirens*, resulting in an olive-gray to black colony with a cottony or woolly texture. Elongate sympodial brown conidiophores are erect and cylindric. Single-celled fusiform conidia ( $2 \times 5\ \mu\text{m}$ ) are produced along the sides of the apical part of the conidiophore, producing flat basal scars. Occasionally phialides without collarettes and annellides may also be seen.<sup>53</sup> *Rhinochadiella atrovirens* frequently has a black yeast *Exophiala* synanamorph. *Rhinochadiella* type conidiation can also be seen as a part of the pleomorphic morphologies of fungi such as *Exophiala* and *Fonsecaea* species. A new species, *R. similis*, isolated from a chronic cutaneous ulcer and identified by molecular characterization, is morphologically indistinguishable from *R. atrovirens* and resides in the *Exophiala spinifera* clade.<sup>16</sup> *Rhinochadiella aquaspersa* is a rare cause of chromoblastomycosis in Central and South America. Growth of mature colonies is often slow but may be achieved in as little as 1 week. Colonies are olive gray to dark gray with a black reverse. Septate, brown-colored hyphae give rise to long, erect, unbranched sympodial conidiophores. Closely spaced pale brown, elliptical, single-celled conidia ( $2 \times 5\ \mu\text{m}$ ) are produced at and near the apex of the conidiophore.





**Figure 14-15** *Ramichloridium mackenziei*. Branching and moniliform hyphae in tissue (Gomori methenamine-silver stain,  $\times 460$ ).

*Ramichloridium mackenziei*. *Ramichloridium mackenziei* is a neurotropic black mould apparently restricted to the Middle East, as all published cases of cerebral phaeohyphomycosis have been from patients who have resided in this region.<sup>54,55</sup> Brain abscess formation is typical with both moniliform and branching dematiaceous hyphae in the aspirated pus (Fig. 14-15). Colonies are velvety in texture and have a dark gray-brown to black, domed surface at maturity, with a black reverse. Septate, pigmented hyphae ( $1.3\text{--}2\text{ }\mu\text{m}$ ) give rise to sympodial conidiophores with relatively few conidia per fertile axis (Fig. 14-16). Small numbers of ellipsoidal, unicellular, brown conidia ( $2.7\text{--}6 \times 4.7\text{--}9.6\text{ }\mu\text{m}$ ) with prominent hila are produced.

*Veronaea botryosa*. *Veronaea botryosa*, an uncommon phaeoid mould, previously thought to be restricted to China and New Guinea, was recently described as an agent of subcutaneous phaeohyphomycosis in a heart transplant recipient in Houston, Texas.<sup>56</sup> Two-celled, brown, smooth-walled conidia with rounded apices and truncate bases ( $2\text{--}4 \times 5\text{--}12\text{ }\mu\text{m}$ ) are formed on long, dark, geniculate conidiophores (Fig. 14-17).

#### Small conidia formed in chains

*Cladosporium* species Although frequently encountered as laboratory contaminants, *C. cladosporioides*, *C. sphaerospermum*, *C. elatum*, and *C. oxysporum* have also been reported as occasional agents of phaeohyphomycosis, as have incompletely identified “*Cladosporium* species.”<sup>57</sup> *Cladosporium bantianum*, *C. carrionii*, and *C. devriesii* are now transferred to the genus *Cladophialophora*.<sup>58</sup>

*Cladosporium* species grow rapidly, producing olivaceous to black velvety colonies. Conidiophores are erect, septate, may contain nodes or swellings, and are often branching. Microscopic features separating *Cladosporium* from *Cladophialophora* species includes prominent “shield cells,” conidia with dark attachment scars (hila), and conidia that are easily dislodged (fragile). Differences in the size and shape of the conidiophores, conidia, and the length of conidial chains differentiate species.

*Cladophialophora* species. Large subunit rRNA sequencing studies<sup>59</sup> and nutritional physiology testing resulted in the taxonomic reclassification of several human pathogens into the genus *Cladophialophora*. The neurotropic species *Cladophialophora*

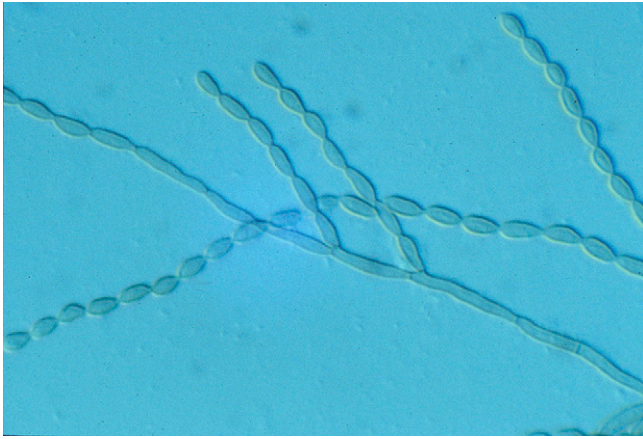


**Figure 14-16** *Ramichloridium mackenziei*. Conidiophores and smooth ellipsoidal conidia with protuberant hila ( $\times 920$ ).

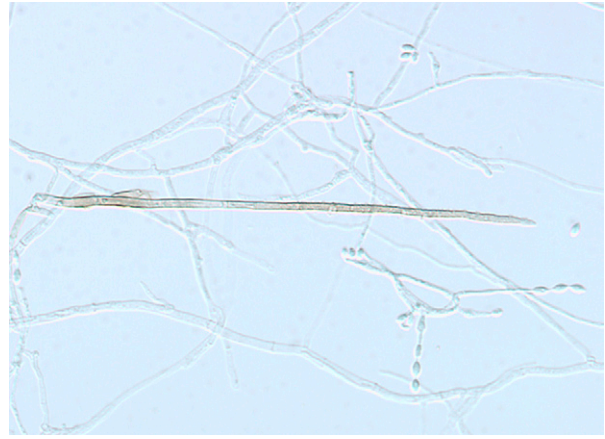


**Figure 14-17** *Veronaea botryosa*. Two-celled, brown, smooth-walled conidia with rounded apices and truncate bases formed on long, dark, geniculate conidiophores.

*bantiana* (formerly *Xylohypha bantiana*, *Cladosporium trichoides*, and *Cladosporium bantianum*) is the most commonly isolated causative agent of cerebral phaeohyphomycosis. As this organism appears to be acquired through the respiratory tract and affects both immunocompetent individuals and solid organ transplant recipients,<sup>60</sup> it should only be handled within a biosafety cabinet. Colonies are olive-gray to brown with olivaceous-black reverse, slightly folded, with moderate



**Figure 14-18** *Cladophialophora bantiana*. Long, infrequently branched chains of lemon-shaped conidia. Note the absence of shield cells and attachment scars (hila) (×920).



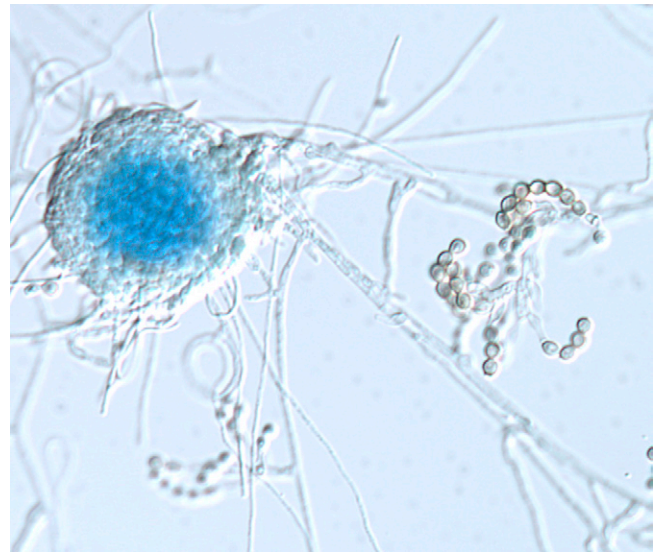
**Figure 14-19** *Acrophialophora fusispora*. Long, dark, young, echinulate conidiophore that will eventually bear conidia at its tip, and chains of hyaline conidia borne from inflated phialides (×920).

growth rate. Good growth at 40°C. Pale olivaceous, ellipsoidal to spindle-shaped conidia (mostly  $2.5\text{--}5 \times 6\text{--}11\text{ }\mu\text{m}$ ) in strongly coherent (non-fragile), infrequently branched chains arise from undifferentiated hyphae (Fig. 14-18). Chlamydoconidia are sometimes seen. Two other infrequently seen neurotropic species include *C. emmonsii* (unpublished data), that fails to grow at 40°C, and *C. modesta*.<sup>61</sup>

*Cladophialophora carrionii* (previously *Cladosporium carrionii*) is among the most frequently isolated organisms associated with chromoblastomycosis, particularly in tropical and subtropical regions, including Australia, South Africa, and South America. Cases of subcutaneous phaeohyphomycosis have also been reported. Colony growth is slow (4 cm at 1 month), and colonies are flat, velvety, olivaceous to black. Hyphae are septate and darkly pigmented. Conidiophores may be lateral or terminal, are variable in size, and give rise to long, branched chains of smooth-walled, elliptical conidia ( $1.5\text{--}3 \times 2\text{--}7.5\text{ }\mu\text{m}$ ) that are more easily disrupted than in other *Cladophialophora* species. The maximum temperature of growth is 36–37°C. Other species infrequently causing human disease include *C. devriesii*,<sup>62</sup> *C. boppii* and *C. arxii*.

*Acrophialophora fusispora*. *Acrophialophora fusispora* is a rapidly growing, thermotolerant, neurotropic mould resembling a *Paecilomyces* species. Colonies are buff or darker with a centrally dark front and reverse. The organism has been the agent of a brain abscess in a leukemic patient<sup>8</sup> and keratouveitis in association with contact lens use, previously identified as *Scedosporium prolificans*.<sup>63</sup> It differs from *Paecilomyces* by having conidia borne in chains from basally inflated phialides produced either directly on hyaline hyphae or along the sides and at the fertile apex of long, dark, echinulate conidiophores anchored by foot cells. Conidia display fine to coarse spiral ornamentation (Fig. 14-19).

*Scopulariopsis* species. Several dark *Scopulariopsis* species are agents of disease, including *Scopulariopsis asperula* and *S. fusca* inciting onychomycosis, and *S. brumptii* in the lung. Various species, such as *S. cinereus* (Fig. 14-20) and *S. paisii*, are anamorphs of *Microascus cinereus*<sup>64</sup> and *M. cirrosus*,<sup>3</sup> respectively. *Scopulariopsis* species produce chains of dry conidia via an annelidic process. They are differentiated mostly by the length of the conidiogenous cells and their annellations, the arrangement



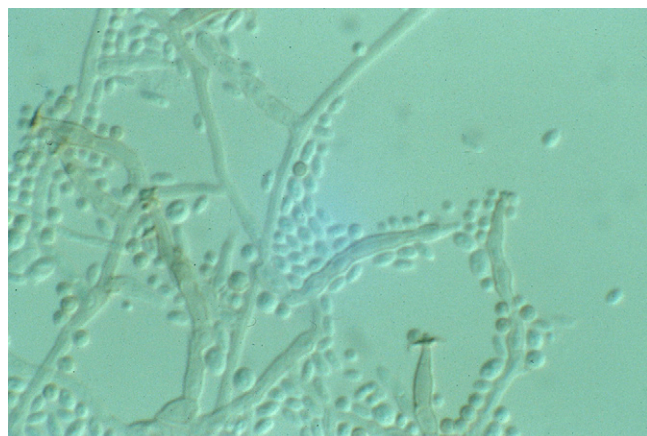
**Figure 14-20** Dematiaceous *Scopulariopsis cinereus*. Young cleistothecium of *Microascus cinereus* and chains of dark annelloconidia of the *Scopulariopsis cinereus* anamorph (×920).

of these cells (single or in scopula), and by the size, shape, and ornamentation of the conidia. Isolates of dark *Scopulariopsis* usually require extended incubation for formation of the *Microascus* teleomorph.

Small conidia mostly produced singly by phialides and/or adelophialides

*Phialophora* species. *Phialophora* species are agents of chromoblastomycosis, mycetoma, and phaeohyphomycosis. They must be distinguished from *Phaeoacremonium* species and from the pleomorphic *Fonsecaea pedrosoi*, which can also display “*Phialophora*”-type conidiation. *Phialophora verrucosa* is the second most common cause of chromoblastomycosis worldwide (after *F. pedrosoi*) and the most common cause in





**Figure 14-21** *Pleurostomophora richardsiae*. Flared collarette is clearly visible on one phialide. Both spherical and allantoid (sausage-shaped) conidia are seen ( $\times 920$ ).



**Figure 14-22** *Phaeoacremonium parasiticum*. Long, tapering, multiseptate phialides that are darker at the base give rise to allantoid (sausage-shaped) conidia ( $\times 920$ ).

North America. Growth is slow, with colonies maturing in approximately 2 weeks. Colony surface color ranges from a dark greenish brown to black, with a black reverse. Colonies may be flat or heaped up and often grow into the agar. Hyphae are septate, brown, and branched. Vase-shaped phialides with deep, flared, darkly pigmented, cuplike collarettes are borne laterally. Subglobose to ellipsoidal conidia ( $1.5\text{--}4 \times 1.5\text{--}4 \mu\text{m}$ ) accumulate at the apices of the phialides. The similar *Phialophora americana* has deeper collarettes. *Phialophora parasitica* has been reclassified as the type species of *Phaeoacremonium*, *Phaeoacremonium parasiticum*, while *P. repens* and *P. richardsiae* have been reclassified as *Pleurostomophora* species.

***Pleurostomophora* species.** *Pleurostomophora*, the anamorph genus of *Pleurostoma*, differs from *Phialophora* by mostly slender rather than flask-shaped phialides that have rather short flaring or inconspicuous collarettes and cylindrical to allantoid (curved) conidia.<sup>65</sup> *Pleurostomophora richardsiae* is an agent of subcutaneous phaeohyphomycosis.<sup>66</sup> Two types of phialides are produced: the more distinctive has a markedly flared, saucer-shaped collarette giving rise to spherical conidia ( $2\text{--}3 \mu\text{m}$ ). The second has an inconspicuous collarette and cylindrical, often curved, conidia ( $1\text{--}3 \times 2\text{--}6.5 \mu\text{m}$ ) (Fig. 14-21). Mature colonies, dark olive-brown, are necessary for the demonstration of both types of conidia. *Pleurostomophora repens* also produces phialides with much less distinct collarettes and bears allantoid (sausage-shaped) conidia collecting in clusters at the apex of phialides. The colonies are normally white initially, later becoming light brown. The maximum growth temperature is  $35^\circ\text{C}$  and above  $40^\circ\text{C}$  for *P. richardsiae* and *P. repens*, respectively.

***Phaeoacremonium* species.** The hyphomycetous genus *Phaeoacremonium* (teleomorph *Togninia*)<sup>67</sup> displays morphologic features between *Acremonium* and *Phialophora*.<sup>68</sup> It differs from the former by darkly pigmented hyphae and conidiophores, and from the latter by inconspicuous collarettes and aculeate conidiogenous cells. Currently, 21 species have been identified on the basis of morphologic and cultural characteristics as well as  $\beta$ -tubulin sequences, and the *Phaeoacremonium* database of all known species can be accessed from the Centraal bureau voor Schimmelcultures website ([www.cbs.knaw.nl/phaeoacremonium.htm](http://www.cbs.knaw.nl/phaeoacremonium.htm)).<sup>7,67</sup>

Although case reports have been published under a variety of names prior to a thorough investigation of the genus, the current list of human pathogens includes *Pm. alvesii*, *Pm. amstelodamense*, *Pm. griseorubrum*, *Pm. kraidenii*, *Pm. parasiticum*, *Pm. rubrigenum*, *Pm. sphinctrophorum*, *Pm. tardicrescens*, and *Pm. venezuelense*.<sup>69</sup> Colonies are pale brown to dark pink and key morphologic features include the presence and size of warts (exudate) on the mycelium, the presence of adelophialides (Type I) and/or phialides (Types II and III), and the size and shape of the hyaline conidia occurring at the apices of conidiogenous cells. *Phaeoacremonium parasiticum* appears to be the most commonly isolated human pathogen, and the species most easily recognized on the basis of its long, Type III phialides and prominent warts<sup>70,71</sup> (Fig. 14-22). Other species generally require sequence confirmation as differences in morphologic features are often subtle.

***Phialemonium* species.** *Phialemonium* was originally described as an anamorph genus intermediate between *Phialophora* and *Acremonium*. Two species are currently recognized: *P. obovatum* and *P. curvatum* (*P. dimorphosporum* = *P. curvatum*).<sup>72</sup> Colonies are moist and spreading. Hyaline conidia collect at the apices of phialides and/or adelophialides (reduced phialides lacking a basal septum). *Phialemonium obovatum* produces obovate (like an upside-down egg) conidia and a diffusible green pigment. It has been an agent of fatal endocarditis in a neonate,<sup>73</sup> lung infection in a bone marrow transplant recipient,<sup>74</sup> and disseminated/osteolytic disease in dogs. Colonies of *P. curvatum* differ from *P. obovatum* by producing buff to yellowish colonies, by sporodochial formation in some strains, and by conidia that may be ellipsoidal, ovoidal or curved. This species has recently been implicated in several cases of hemodialysis-associated endovascular infections,<sup>75</sup> endocarditis and endophthalmitis linked to contaminated intracavernous penile injections for the treatment of impotence,<sup>76,77</sup> and arthritis following intraarticular injection of corticosteroids.<sup>78</sup>

***Lecythophora* species** Colonies of *Lecythophora* species are distinguished from *Phialemonium* and *Phaeoacremonium* species by being pink to salmon with an orange reverse. Conidiogenous cells are predominantly adelophialides exhibiting very narrow collarettes. Conidia ( $3\text{--}6 \times 1.5\text{--}2.5 \mu\text{m}$ ) are smooth-walled, hyaline, ellipsoidal, cylindrical or allantoid and aggregate



in clusters at the apices of conidiogenous cells. *Lecythophora mutabilis* is distinguished from *L. hoffmannii* by brown chlamydoconidia and slightly larger conidia. Organisms are agents of endophthalmitis,<sup>79</sup> sinusitis,<sup>80</sup> and endocarditis.<sup>81</sup>

Small conidia formed on denticles or ampulliform swellings

*Ochroconis gallopavum*. *Ochroconis gallopavum* (*Ochroconis gallopava*) is a phaeoid, velvety mould that tends to be more brownish than gray-black, and produces a red to mahogany diffusible pigment. The organism has a predilection for the central nervous system, displays good growth up to 45°C, and fails to grow on media containing cycloheximide. Hyaline to pale brown, clavate, two-celled conidia measuring 11–18 × 2.5–4.5 µm and constricted at the septum are borne at the tips of denticles. These features separate *O. gallopavum* from other non-neurotropic *Ochroconis* species such as *O. constricta* and *O. humicola*. Recent reports include dissemination in the setting of advanced HIV,<sup>82</sup> leukemia,<sup>83</sup> and solid organ transplants.<sup>84</sup>

*Myceliophthora thermophila*. Colonies of *Myceliophthora thermophila* are pale brown and powdery to granular with an ill-defined margin. This thermophilic species, an agent of disease in cultivated mushrooms and common in thermal, decaying plant material, produces more luxurious colonies at elevated temperatures (up to 48°C). Single-celled conidia (4.5–11 × 3–4.5 µm), initially hyaline and smooth but rough-walled and brown at maturity, are borne terminally or laterally, singly or in small groups, from ampulliform swellings. This organism's apparent predilection for the vascular system, as evidenced by reports of fatal disseminated disease,<sup>85,86</sup> underscores its pathogenic potential. A case of severe osteomyelitis following an injury to the knee with a pitchfork contaminated with barnyard matter was recently reported.<sup>87</sup>

#### Two-celled arthroconidia

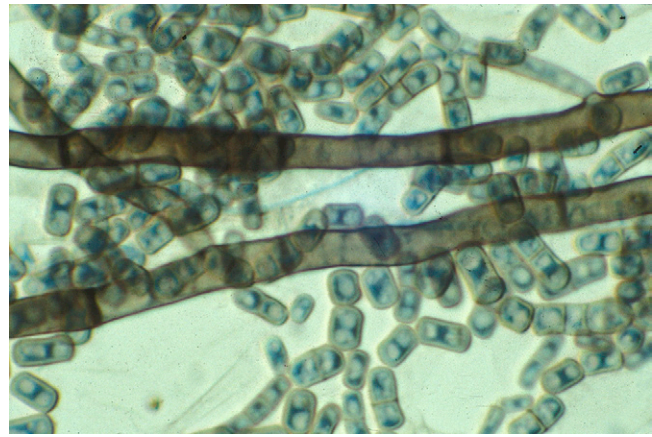
See discussion and description of the *Scytalidium dimidiatum* synanamorph of *Nattrassia mangiferae* under the section on coelomycetes, below.

Isolates usually sterile (fail to produce reproductive propagules)

*Madurella* species. See discussion and description of *Madurella mycetomatis* and *Madurella grisea* under the section on dematiaceous agents of mycetoma, below.

### Coelomycetes

*Nattrassia mangiferae*/*Scytalidium dimidiatum*. This organism, known by both its coelomycetous form, *N. mangiferae*, and its arthroconidial synanamorph, *S. dimidiatum*, is a common agent of dermatomycoses and onychomycosis in patients living in or immigrating from tropical areas. There have been more recent reports of invasive disease in immunocompromised hosts.<sup>88</sup> Colonies are black and woolly and fill the plate or tube within 3–4 days. One- and two-celled arthroconidia (4–16.5 × 8.5 µm), not separated by disjunct cells, are produced from dark, wide (up to 10 µm in diameter) hyphae (Fig. 14-23). After extended incubation (sometimes requiring banana peels), versicolored (darker middle cells) conidia (10–16 × 3.5–6.5 µm) are produced from phialides



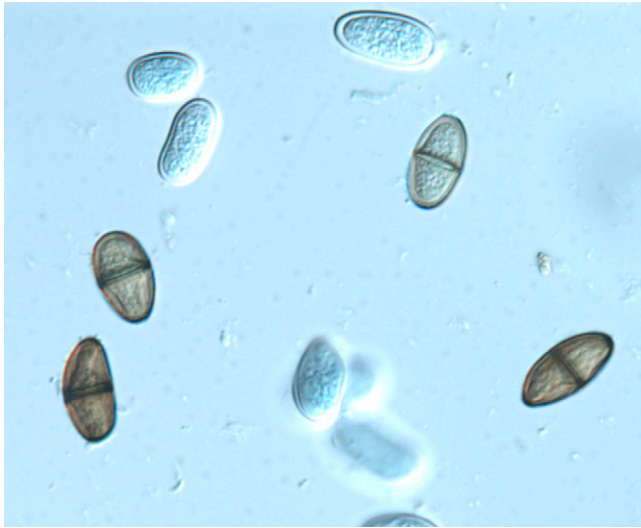
**Figure 14-23** *Scytalidium dimidiatum* synanamorph of *Nattrassia mangiferae*. Dark septate hyphae and thick-walled arthroconidia (×920).

within multilocular pycnidia. The hyaline variant, previously known as *S. hyalinum*, is now considered synonymous with *S. dimidiatum*.<sup>89</sup>

*Phoma* species. Several *Phoma* species have been reported to cause human disease, primarily cutaneous.<sup>3</sup> Colonies are greenish gray to brown; some species may develop red or pinkish pigmentation. The growth rate varies widely depending on the species. Dark, globose to subglobose to pyriform pycnidia with single or multiple ostioles occur on or immersed in the agar and may form singly or in aggregates. The hyaline conidiogenous cells lining the inner walls of the pycnidia produce their conidia by percurrent proliferation<sup>90</sup> which are then extruded in slimy masses. The variably shaped conidia are unicellular and hyaline. Alternarioid chlamydoconidia are seen in some species. Boerema et al have recently provided an excellent revision and identification manual for the genus.<sup>90</sup> Closely related genera include *Pleurophoma* and *Pleurophomopsis*, an agent of maxillary sinusitis.<sup>91</sup> Separation of these genera is best performed in a reference laboratory.

*Coniothyrium*/*Microsphaeropsis*/*Paraconiothyrium* species. *Coniothyrium fuckelii* is a known plant pathogen that has also reportedly caused infections in immunocompromised individuals. Colonies are light brown with a darker reverse; a wine-colored diffusible pigment may also be observed. The growth rate is moderate. Pycnidia (180–300 µm) are brown and subspherical. In addition to conidiogenous cells, sterile hyphal elements called paraphyses line the inner walls of the pycnidia. The conidia are ovoid, smooth-walled, and pale brown. Most authorities would place this organism in the genus *Microsphaeropsis*. Other *Microsphaeropsis* species causing disease include *M. arundinis*<sup>92</sup> and *M. olivaceae*.<sup>93</sup> A new genus of *Coniothyrium*-like fungi, *Paraconiothyrium*,<sup>94</sup> has been recently described. Identification of these similar/taxonomically related fungi is facilitated by molecular characterization and best handled by individuals with expertise in these genera.

*Lasiodiplodia theobromae*. Most frequently seen as an agent of keratitis, *Lasiodiplodia theobromae* produces dark, large (up to 5000 µm), sometimes setose, ostiolate, variably shaped pycnidia after extended incubation (occasionally requiring up to 10 weeks). Large conidia (20–30 × 10–15 µm) are initially



**Figure 14-24** *Lasiodiplodia theobromae*. Immature (hyaline, one-celled) and mature (dark, striated, two-celled) conidia (×920).

aspetate and hyaline, later becoming single-septate, dark, and longitudinally striated (Fig. 14-24).<sup>95</sup>

## Ascomycetes

The organisms described here (as well as *Leptosphaeria* species discussed under agents of mycetoma, below) are distinguished by the formation of complex fruiting structures known as ascomata under routine culture conditions, and the production of various types of ascospores.

**Chaetomium species.** Although *Chaetomium* is a large genus, only a few species have been implicated in human disease. Species identification is based upon temperature tolerance, and the size and shape of the perithecia, the setae or hairs covering the perithecia, and the mature ascospores. *Chaetomium globosum* grows at 35°C but fails to grow at 42°C. It is an agent of onychomycosis, cutaneous lesions, peritonitis, and a case of subcutaneous phaeohyphomycosis.<sup>96</sup> Setae are coiled and brown ascospores subglobose to lemon-shaped, hence the species epithet “globosum.” Three species grow at 42°C, are neurotropic, and agents of cerebral phaeohyphomycosis: *C. atrobrunneum*, *C. strumarium*, and *C. perlucidum*.<sup>97</sup> *Chaetomium strumarium* (*Achaetomium strumarium*) produces a pink to brown diffusing pigment and pink exudate. See Barron et al for differentiation of species.<sup>97</sup>

**Microascus species.** Another perithecial-forming genus, *Microascus*, is being seen as an invasive agent in bone marrow transplant recipients. Three different species of *Microascus*, all having a dark *Scopulariopsis* anamorph, have been reported. Species are distinguished primarily by the size of their perithecia and length of their perithecial necks, as well as the size and shape of the reddish-brown ascospores. Ascospores are extruded from perithecial openings in a long cirrus (like toothpaste from a tube) and are orange segment-shaped, heart-shaped, or triangular-shaped in *M. cinereus*,<sup>64</sup> *M. cirrosus*,<sup>98</sup> and *M. trigonosporus*,<sup>99</sup> respectively. Extended incubation is generally required for perithecial development in phaeoid *Scopulariopsis* species.

## Dematiaceous agents of mycetoma

***Madurella mycetomatis*.** *Madurella mycetomatis* is the most common cause of eumycotic mycetoma worldwide, causing disease predominantly in South America, Africa, and India. Granules (0.5–5 mm diameter) are reddish brown or black and hard and are composed of hyphae embedded in a brown, cementlike matrix. Colony growth is faster at 37°C than at 25 or 30°C, and growth occurs up to 40°C. The macroscopic appearance is variable. Colonies are white initially, later becoming yellow, brown or olivaceous; a brown diffusible pigment may be produced. The texture varies from glabrous to velvety, and they may be flat or heaped. On SDA, only sterile septate hyphae with few chlamydoconidia are produced. If nutritionally deficient media are used, tapering phialides (3–15 µm long) may be produced, which give rise to pyriform to oval conidia (3–4 µm). Black masses of hyphae called sclerotia (750 µm diameter) may also be seen in mature cultures. *Madurella mycetomatis* is differentiated from *M. grisea* by its ability to grow at temperatures up to 40°C and its inability to assimilate sucrose.

***Madurella grisea*.** *Madurella grisea* is a cause of “black grain” mycetoma in India, Africa, Central and South America, and rarely in the United States. Granules (0.3–0.6 mm diameter) are black and soft. As with *M. mycetomatis*, a brown cementlike material is seen in the periphery of the granules in tissue sections. Colonies are slow growing, with olive brown to black surface coloration and black reverse; a red-brown diffusible pigment may be produced. Colony surface is velvety or smooth and furrowed. Pigmented hyphae (1–3 µm, but sometimes 3–5 µm with beadlike swellings) are septate and sterile. Chlamydoconidia are seen rarely. Isolates producing pycnidia have been reported; these cannot be distinguished from *Pyrenochaeta mackinnonii*.

***Leptosphaeria* species.** *Leptosphaeria senegalensis* and *L. tompkinsii* cause mycetoma in West Africa (specifically Senegal and Mauritania) and India. Granules (0.5–2 mm) are black and hard. In tissue sections, the central part consists of hyphae, and a black cementlike substance is seen at the periphery. Both organisms grow rapidly, producing brown to gray colonies. Black, spherical to subspherical ascomata lack ostioles. Asci are eight-spored. The two species are distinguished by microscopic features of their ascospores.

***Pyrenochaeta* species.** Both *P. romeroi* and *P. mackinnonii* are known to be agents of mycetoma in Africa, India, and South America. Granules (0.2–0.6 µm) are black and soft, without a cementlike matrix in histologic sections. Cultures grow rapidly. The colony surface is gray with a lighter margin and black reverse and without diffusible pigment. The septate, branched hyphae may be either hyaline or pigmented. *P. romeroi* produces pycnidia (40–100 × 50–160 µm) on nutritionally deficient media that bear elliptical pycnidiospores (1 × 1.5 µm). The pycnidia closely resemble those found in some cultures of *Madurella grisea*. The precise identification of all agents of black-grain mycetoma is facilitated by ITS sequencing.<sup>100</sup>

The most common cause of eumycotic mycetoma in North America is *Pseudallescheria boydii*. In this work, *P. boydii* is addressed in the chapter on agents of hyalohyphomycosis. Although this organism develops dark pigmentation in culture, it appears similar to agents of hyalohyphomycosis in tissue and will not be further described here. Some dematiaceous agents of mycetoma also cause phaeohyphomycosis and are discussed

elsewhere in the chapter. These include *Curvularia* species (*C. lunata* and *C. geniculata*) and *Exophiala jeanselmei*.

## Clinical aspects

### Chromoblastomycosis

Chromoblastomycosis is a chronic infection of the skin and subcutaneous tissues caused by one of several dematiaceous fungi, which is distinguished by the unique finding of muriform “sclerotic bodies” on microscopic examination of material from lesions. This disease entity is thought to have been first recognized in 1911 by Pedrosa, but because of a 9-year delay in publication, the first published report was written by Rudolph in 1914.<sup>101</sup> Medlar published the first description of an etiologic agent the following year.<sup>102</sup> Several alternative disease names have been used in the literature, including chromomycosis, Pedrosa’s disease, Fonseca’s disease, Gomes’s disease, blastomycose nigra, verrucous dermatitis, and figuera. The term “chromoblastomycosis” is preferred, because it was the name originally used for this disease process; use of “chromomycosis” is discouraged because of its previous use as a descriptor for any disease caused by dematiaceous fungi (similar to the current usage of phaeohyphomycosis).<sup>103</sup>

#### Epidemiology

Chromoblastomycosis has a worldwide distribution but has been reported most frequently from tropical or subtropical locations. Particularly high incidence rates have been reported from Brazil,<sup>104</sup> Madagascar,<sup>105</sup> and Costa Rica; the causative organisms have been isolated from soil and decaying vegetation in these and other high-prevalence areas. There has been a male predominance in most series,<sup>101</sup> and many affected individuals work outdoors without footwear. A recognized penetrating injury at the involved site has occasionally preceded the development of the chromoblastomycosis lesion, but more commonly a traumatic event is not recalled, and it is thought that infection in these cases occurs through minor breaks in skin integrity. Person-to-person spread of chromoblastomycosis has not been documented.

#### Clinical features

Lesions develop most commonly on the distal lower extremities, a location compatible with exposure of damaged skin to soil. The primary skin lesion is a small papule that gradually enlarges over weeks to months to form a superficial nodule with an irregular, friable surface. Lesions continue to evolve, often over many years, and at a given time may have morphologic features of one or more of the five types of chromoblastomycosis lesions described by Carrion.<sup>106</sup> Tumorous lesions are larger than nodular lesions, with raised surface projections that may be covered by crusting and epidermal debris; these lesions can become very large, and their surface texture has been compared with that of cauliflower. Verrucous lesions are warty and hyperkeratotic. Plaque lesions, the least common type, are flat, reddish, and scaly. Cicatricial (scarring) lesions have irregular borders and expand at their periphery with central healing and scarring. “Black dots” may be observed on the surface of lesions; samples of material from these areas are particularly useful for microscopic examination.

Although lesions may be painful, particularly if they become secondarily infected, in most cases they are relatively asymptomatic. Involved skin can also be pruritic. Autoinoculation from scratching or from superficial lymphatic spread may result in satellite lesions. Lymphadenitis and consequent lymphedema can develop as a result of secondary bacterial infection. Hematogenous spread to other organs including the central nervous system has been reported but is rare; squamous cell carcinoma has also been seen as a complication of long-standing chromoblastomycosis lesions.

#### Diagnosis

Although the appearance and location of the skin lesions and the typically chronic history may be most suggestive of chromoblastomycosis, the differential diagnosis can include tuberculosis, mycetoma, leprosy, blastomycosis, and cutaneous leishmaniasis.<sup>101</sup> Examination of material from the lesions is therefore necessary to make a definitive diagnosis in most cases, and culture should be performed to determine the causative agent. By definition, chromoblastomycosis is characterized by the presence of unique “sclerotic bodies” in involved tissues. Sclerotic bodies (5–12  $\mu\text{m}$  in diameter) are chestnut brown, round, thick-walled structures that are muriform (they have both horizontal and vertical septa). Alternative descriptive terms in usage include Medlar bodies, muriform cells, and “copper pennies.” These structures were at one time thought to be budding yeast cells, resulting in the name chromoblastomycosis. They are now considered to be a form of the fungus arrested between the yeast and hyphal morphologies, which develops as a result of acidic conditions in the involved tissues and possibly other undefined local factors.<sup>107</sup>

The simplest first step in diagnosis is to examine a potassium hydroxide preparation of material scraped from the surface of the lesion, preferably from an area containing “black dots.” Sclerotic bodies may be detected on this type of direct microscopic examination, but more often biopsy with histologic examination is necessary. The pathology is similar irrespective of the causative organism. The epidermis is markedly thickened (termed pseudoepitheliomatous hyperplasia) and may contain microabscesses in regions infiltrated by polymorphonuclear leukocytes. In addition to similar microabscesses, the dermis contains granulomas consisting of multinucleated giant cells and epithelioid cells. The tissue surrounding these focal abnormalities shows a mixed cellular infiltrate; marked fibrosis can be seen with older lesions. Sclerotic bodies are seen both within giant cells or macrophages and extracellularly in microabscesses. Hyphae may also be seen in the epidermis.

Because the causative agents cannot be distinguished on the basis of histologic features, culture of lesion material is necessary. The specimen should be plated on culture media both with and without antibiotics because of the possibility of bacterial contamination, and inoculated plates should be incubated at both 25°C and 30°C. In most cases, colonies are formed within 2 weeks; cultures should be held for 4 weeks before being reported as negative. Serodiagnostic techniques and skin testing have not been found to be useful to date outside research settings.

#### Microbiology

Five dematiaceous organisms have been traditionally recognized as the causative agents of chromoblastomycosis: *Fonsecaea pedrosoi* (most common), *Fonsecaea compacta* (possibly



a morphologic variant of *F. pedrosoi*<sup>108</sup>), *Cladophialophora* (*Cladosporium*) *carrionii*, *Phialophora verrucosa*, and *Rhino-cladiella aquaspersa*.<sup>109</sup> More recently, published cases have been attributed to infection with *Exophiala jeanselmei*, *Exophiala spinifera*, and the newly described organism *Fonsecaea monophora*.<sup>108</sup> All of these causative agents produce similar slow-growing dark brown or olivaceous to black colonies with a velvety texture. Careful study of microscopic morphology is therefore key to determining which organism has caused a given infection.

### Treatment

Therapeutic approaches to chromoblastomycosis include surgery and non-surgical physical modalities for localized lesions and antifungal medications for more extensive disease. Surgical excision is the best treatment for small or early lesions but because of the typically late clinical presentation, the extent of tissue involvement most often precludes this approach. Liquid nitrogen cryotherapy,<sup>110</sup> direct application of heat, and laser photocoagulation are alternative approaches for less extensive lesions that have been used with some reported success. Electrocautery and curettage could result in local spread or dissemination and are therefore discouraged.<sup>109</sup>

Systemic antifungal therapy is generally required for more extensive infections, but unfortunately the results of therapy have often been disappointing, with partial responses and relapses after withdrawal of therapy being common outcomes. Antifungals have been used as single agents, in a variety of combinations, and in conjunction with physical measures.<sup>111</sup> The agents that have been studied in published case series include thiabendazole, 5-fluorocytosine, amphotericin B, ketoconazole, fluconazole, terbinafine, itraconazole and posaconazole. Because the outcomes of therapy with the earlier agents were disappointing, recent reports have focused on studies of therapy with newer agents including itraconazole,<sup>112-116</sup> terbinafine,<sup>117-119</sup> and posaconazole.<sup>120</sup> Itraconazole dosing has ranged from 100 to 400 mg/day alone or when combined with cryotherapy or terbinafine. “Pulse” itraconazole therapy (400 mg/day for 1 week of every month) has been reported to be successful for 5/6 patients with chromoblastomycosis caused by *Fonsecaea pedrosoi*<sup>114</sup> and alternate-week itraconazole and terbinafine have also been used with some success in a few refractory cases.<sup>115</sup>

## Mycetoma

Mycetoma (also sometimes referred to as “Madura foot” or “maduromycosis”) is a chronic infection involving cutaneous and subcutaneous tissues that is characterized by draining sinuses that extrude masses of the infecting organism termed “granules,” “grains” or “sclerotia.” Mycetoma may be caused either by a fungus (eumycotic mycetoma) or by aerobic actinomycetes (actinomycotic mycetoma); only the former will be considered in this discussion. Use of the label mycetoma to describe a localized “fungus ball” (such as in a pulmonary cavity or a paranasal sinus) is believed to be an inappropriate and potentially confusing usage of the term.<sup>103, 121</sup>

Mycetoma has long been recognized as a distinct disease entity, with the earliest known written reference appearing in an Indian religious book, *Atharva Veda*.<sup>121</sup> The disease was later apparently described by missionaries in India in the

18th century, and in the first medical publications the name “Madura foot” was proposed because it was the term used to describe the disease in India’s Madura district. In the 1860s, the involvement of fungal organisms as causative agents was recognized, and the name mycetoma was first used. By the end of the 19th century, fungi and actinomycetes were recognized to cause black-grain and light-grain mycetoma respectively.

### Epidemiology

Although cases of eumycotic mycetoma have been reported in essentially a worldwide distribution, infection rates are highest in tropical and subtropical countries. Particularly high rates are reported from the Sudan, India, Pakistan, Somalia, and parts of South America. The organisms responsible for causing disease can vary dramatically from region to region. For example, 50% of cases in Pakistan are caused by *Madurella mycetomatis* whereas *Pseudallescheria boydii* is the most common agent in temperate areas, including the United States. The most important factor responsible for this regional variation is thought to be the annual amount of rainfall.

Infection results from traumatic inoculation of soil fungi into the skin, usually with a thorn or other foreign object. Approximately 70% of mycetoma cases involve the foot and 15% affect hands, but any part of the body can be involved; this distribution reflects the relative frequency of trauma at these anatomic sites. A male predominance of infection in a 5:1 ratio has been reported. This probably represents a true difference in susceptibility, because this difference is seen even in areas where women and men do similar amounts of outside work.<sup>122</sup>

### Clinical features

Patients usually present for medical attention months to years after the inciting traumatic event. The clinical hallmarks of mycetoma are swelling, draining sinuses, and granules, which are microcolonies of the etiologic agent that are extruded through the sinuses. The primary lesion consists of a small non-tender subcutaneous nodule, which may develop up to years after the inciting traumatic event. The lesion gradually enlarges, becomes softer and ruptures to the surface, forming sinus tracts, while at the same time also spreading to simultaneously involve deeper tissues. At least several months are required for the formation of sinus tracts in eumycotic mycetoma; disease progression may be faster for actinomycotic disease. Sinus formation is a dynamic process, with fresh sinuses opening near areas that have temporarily closed after discharging exudate and granules. The surfaces of sinus openings of eumycotic mycetomas are flush with the skin surface, distinguishing them from the raised openings of sinuses caused by actinomycetes. Exudate can be serous, serosanguinous or seropurulent; granules range in size from 0.3 to 5 mm, and in most cases of eumycotic mycetoma they are brown or black. The overlying skin may become attached to areas of subcutaneous inflammation. The resulting swelling stretches the skin, which becomes smooth and shiny.

Eventually the granulomatous inflammation will extend to involve bone and may cause destructive lesions, but bone involvement is less extensive in eumycotic compared with actinomycotic mycetoma. Ligaments may also be involved, but muscle and tendons normally remain intact in eumycotic mycetoma. A chronically infected foot eventually becomes

shortened because of bone destruction and plantar fibrosis and the forces exerted by intact tendons. In the absence of secondary bacterial infection, there is little pain and there are no systemic symptoms. Spread beyond the subcutaneous tissues has not been observed in cases caused by dematiaceous fungi.

### Diagnosis

An appropriate therapeutic approach requires that mycetoma be distinguished from botryomycosis (a bacterial infectious process that may be clinically similar)<sup>123</sup> and “pseudomycetoma,” an unusual form of dermatophyte infection.<sup>124</sup> The differential diagnosis also may include sporotrichosis, chromoblastomycosis, coccidioidomycosis and yaws, depending on the geographic location.

The clinical finding of compatible skin lesions with sinuses discharging granules is suggestive of the diagnosis, particularly in an endemic area. Characterization of granules by their color and by the size of the filaments composing them (0.5–1 µm diameter suggests actinomycetes, 2–6 µm fungi)<sup>125</sup> on direct microscopic examination of potassium hydroxide preparations can aid in differentiating between actinomycotic and eumycotic mycetoma; culture of the organism and histopathologic examination are recommended for definitive diagnosis of mycetoma. Culture of expressed granules themselves most often is not helpful, because the causative organism may not be viable and bacterial contamination is almost invariably present. Culture and histologic examination should therefore be performed on deep or excisional biopsy specimens.

Hematoxylin and eosin (H&E) or methenamine silver is used to stain tissues from most fungal mycetomas. Granules are seen either within sinus tracts or in the tissues, and those caused by dematiaceous fungi are darkly pigmented even when unstained. In tissues they are frequently seen in the center of an abscess surrounded by neutrophils. Fibrosis and granulomatous inflammation composed of macrophages, epithelioid cells, and multinucleated giant cells are seen around the abscesses. The latter two findings can also be seen in other disease processes, however, and true granules with associated suppuration are required for a pathologic diagnosis of mycetoma.

Specimens containing dematiaceous fungi should be planted on Sabouraud's agar with yeast extract and without cycloheximide and incubated at 25°C. Growth is typically slow; plates should be kept 6–8 weeks before being considered negative. Subculture to cornmeal or potato dextrose agar may enhance production of conidia, aiding definitive identification. PCR followed by sequencing of the product may prove useful for identification of isolates that are slow to produce conidia.<sup>126</sup>

### Microbiology

Eumycetoma has been reported to be caused by a diverse group of fungal organisms.<sup>127</sup> The common dematiaceous agents of mycetoma include *Madurella mycetomatis*, *Madurella grisea*, *Exophiala jeanselmei*, *Leptosphaeria senegalensis* and *Pyrenochaeta* species.

### Treatment

Although still frequently practiced in some areas, surgical treatment alone results either in early recurrence due to incomplete resection or in unnecessarily large tissue defects. For these reasons, medical or combined medical and surgical therapies are

recommended. The combined approach involves treating the patient with antifungal drugs until evidence of clinical response (usually 1–2 months) and for at least 6–12 months after surgical removal of the lesion.

Antifungal drugs must be used for even more prolonged periods when medical therapy alone is used. In the very few reported cases of treatment with fluconazole, failures and relapses have been common. The results of amphotericin B for mycetoma due to *E. jeanselmei*, *M. mycetomatis* or *M. grisea* have been mixed.<sup>128</sup> Ketoconazole (200 mg twice daily) was administered to for 9–36 months in 50 patients with infection due to *M. mycetomatis* in one study, with encouraging results: 72% were either cured or markedly improved, 20% showed some improvement, and 8% showed no improvement or deteriorated.<sup>129</sup> Itraconazole has also been used with success. Some experts report outcomes superior to those observed with ketoconazole therapy,<sup>130</sup> but itraconazole is thought to be less effective than ketoconazole in cases caused by *M. mycetomatis*.<sup>131</sup> Promising treatment responses have recently been reported for patients treated with terbinafine (27 patients),<sup>132</sup> voriconazole,<sup>133</sup> and posaconazole (six patients with refractory mycetoma).<sup>120</sup>

## Phaeohyphomycosis

Translated into English, the term “phaeohyphomycosis” (from the Greek root *phaios*, meaning dusky or dark-colored) means “condition of dark hyphal fungus.” It was first proposed by Ajello et al in 1974<sup>134</sup> as a descriptor for “infections caused by hyphomycetous fungi that develop in the host tissues in the form of dark-walled dematiaceous septate mycelial elements.” It was intended that the use of this new classification would allow a clinical grouping of infections caused by darkly pigmented fungi while reducing the confusion caused by the introduction of many new disease names based on ever-changing mycologic nomenclature. This new designation was also introduced to separate the clinically distinct disease process of chromoblastomycosis from the remainder of infections caused by dematiaceous fungi, which had been inappropriately included in the term “chromomycosis” since 1935.<sup>135</sup>

Ironically, because of an evolution in the accepted definition of phaeohyphomycosis, the term itself has become a source of some confusion in medical and mycologic literature. As originally proposed, only mycelial fungi in the form-class Hyphomycetes of the form-division Fungi Imperfecti could be agents of phaeohyphomycosis. The definition was broadened by Ajello et al in 1981<sup>136</sup> to include dematiaceous members of the form-class Coelomycetes of the form-division Fungi Imperfecti and members of the division Ascomycota. McGinnis et al<sup>137</sup> subsequently further redefined the term to include infections caused by all agents appearing in tissue as dematiaceous yeast cells, pseudohyphae-like elements, septate hyphae or any combination of these forms. It should be emphasized that although dematiaceous fungi by definition contain melanin in their cell walls, pigmentation is not always visible on tissue sections when standard stains are used. In such cases, a special melanin stain such as the Fontana–Masson stain is helpful in revealing the dematiaceous nature of the pathogen.<sup>138,139</sup>

Chromoblastomycosis and eumycotic mycetoma have traditionally been considered separately from phaeohyphomycosis because of the unique pathologic features that allow their

distinction from the rest of the infections caused by dematiaceous fungi. This has remained the common usage in the literature despite a recommendation of the second nomenclature committee of the International Society for Human and Animal Mycology (ISHAM) that the term “phaeohyphomycosis” should be a generic term for any mycosis involving a dematiaceous fungus.<sup>3</sup> Without considering the agents of chromoblastomycosis or mycetoma, the list of currently known causative organisms includes over 100 different species in at least 60 genera;<sup>140</sup> this number will undoubtedly continue to increase as new cases are reported in the literature.

Phaeohyphomycosis has been divided into four disease categories by Fader and McGinnis: superficial, cutaneous and corneal, subcutaneous, and systemic.<sup>109</sup> Revankar further divided systemic disease into several categories including allergic disease, pneumonia, brain abscess and disseminated infection in his recent review of the topic.<sup>141</sup> combination of these approaches to organization of the disease entities will be used in this chapter.

### Superficial

**Tinea nigra.** Tinea nigra (synonyms tinea nigra palmaris, keratomycosis nigricans, and others) is a superficial cutaneous fungal infection caused by *Hortaea werneckii* (*Phaeoannellomyces werneckii*, *Exophiala werneckii*, *Cladosporium werneckii*). In the strictest sense, the term “tinea” should be restricted to disease processes caused by dermatophytes, but continued use of the name tinea nigra was recommended by the second ISHAM nomenclature committee because of its long history of use.<sup>103</sup> This infection is endemic in tropical and subtropical coastal regions in the Caribbean, Central and South America, Asia, and Africa; cases have also been reported from southeastern US coastal states and Europe. Children and young adults are most frequently affected, and most infections are reported from immunocompetent individuals.

The usual clinical presentation of tinea nigra is the asymptomatic development of a single painless sharply demarcated brown to black macule. Tinea nigra is typically located on the palmar surface of the hand or finger (more commonly) or on the plantar surface of the foot, but other sites including the neck or trunk can be involved. The lesion gradually enlarges, with the darkest pigmentation found at the periphery, rarely with associated areas of scaling. The infection is confined to the stratum corneum, and therefore normally does not elicit an inflammatory reaction.

Tinea nigra must be distinguished from other causes of hyperpigmented lesions,<sup>142</sup> the most important of these being the acral lentiginous form of malignant melanoma. This is most rapidly achieved by direct microscopic examination of scrapings from the lesion with potassium hydroxide. Brown to olivaceous septate branching hyphae and elongate budding cells with some chlamydoconidia are seen. Culture should also be performed for confirmation.

Topical therapies are the accepted treatment for tinea nigra. Keratolytic agents such as Whitfield's ointment, other salicylic acid preparations, and tincture of iodine are among the most efficacious treatments. Other topical agents used with success have included terbinafine, 10% thiabendazole, and the imidazole agents miconazole, econazole, clotrimazole, and oxiconazole.<sup>142,143</sup> Oral griseofulvin has been shown to be ineffective; successful oral therapy has been reported with

ketoconazole and itraconazole.<sup>142</sup> Relapse normally does not occur after effective therapy, although recurrences may occur as a result of reexposure.

**Black piedra.** Black piedra is a fungal infection of the hair shafts of humans and animals caused by *Piedraia hortae*. The source of the organism appears to be the soil; in some cases, exposure results from use of plant oil in hair.<sup>142</sup> Infection occurs predominantly in the tropical climates of Central and South America, southeast Asia, and the South Pacific islands.

The clinical presentation is the finding of multiple 1–2 mm hard, darkly pigmented oval nodules adherent to hair shafts. Infection is normally restricted to scalp hair but may involve hair at any site. Multiple nodules may be present on a single hair, weakening the hair shaft and possibly resulting in breakage. Patients do not experience pruritus and they are otherwise asymptomatic apart from the cosmetic effects of the nodules; in fact, some cultural groups in endemic areas consider this infection to be cosmetically appealing and encourage practices that result in its development.<sup>144</sup>

Black piedra can be distinguished from pediculosis, white piedra (caused by *Trichosporon* species), tinea capitis and other similar conditions by examining individual hairs in a KOH preparation using light microscopy. The black piedra nodules are composed of aligned, dichotomously branching hyphae surrounding a cement-filled stroma with areas containing asci, each of which holds eight fusiform, curved ascoconidia. *Piedraia hortae* grows on routine mycologic culture media.

The simplest treatment is to simply cut the involved hairs. Topical treatments including salicylic acid, benzoic acid or mercury perchloride (1:2000) are also effective. Oral terbinafine has been reported to be effective in a single case.<sup>145</sup> Relapse appears to be common irrespective of the treatment used.

### Cutaneous and corneal

**Dermatomycosis and onychomycosis.** Some agents of phaeohyphomycosis are capable of causing dermatomycosis and onychomycosis similar to those caused by dermatophytes. These infections, like those classified as “superficial,” involve only keratinized tissues but the degree of tissue damage and the associated immune response are greater, resulting in a different clinical presentation. Dark pigmentation of the nail and an associated paronychia<sup>146</sup> can be clues leading to suspicion of involvement of dematiaceous organisms in onychomycosis; cutaneous phaeohyphomycosis is clinically indistinguishable from dermatomycosis. *Exophiala jeanselmei* has been implicated in onychomycosis<sup>147</sup> and *Alternaria* species and *Nattractia mangiferae*/*Scytalidium dimidiatum* may cause both types of infection.<sup>146</sup> Because of resistance to some of the antifungals used to treat dermatophyte infections, infection with these organisms may be a cause of “recalcitrant dermatomycoses.”<sup>109</sup>

Dematiaceous hyphae may be seen in nail scrapings examined in 30% KOH containing 40% dimethyl sulfoxide, but the presence of hyaline-appearing hyphae does not rule out these organisms. Culture confirmation is required. At least one medium that does not contain cycloheximide should be used, because the growth of *Nattractia mangiferae* is inhibited in its presence.

Treatment can be frustrating, with inconsistent results being seen with the currently available antifungals. Whitfield's ointment may be effective for cutaneous disease, and



itraconazole and terbinafine are the agents most frequently used for systemic therapy of nail disease.<sup>141</sup>

### Subcutaneous

Subcutaneous phaeohyphomycosis, which also appears in the literature under the label phaeomycotic cyst (and earlier as phaeosporotrichosis, among other names), is an uncommon localized infection of the deep dermis and subcutaneous tissues caused by dematiaceous fungi. It is the most frequently reported of the various clinical forms of phaeohyphomycosis. Infection is thought to result from traumatic implantation of the causative fungal organism into the subcutaneous tissue. Although the inciting trauma is not always recalled because of the typically chronic disease course, the propensity for involvement of the distal extremities and the finding in some cases of wood splinters in tissue are supportive of this mode of infection. This form of phaeohyphomycosis is more common in warm climates and immunocompromised individuals are at increased risk, but otherwise no particular group appears to be predisposed. Person-to-person spread does not occur.

The usual clinical presentation is the asymptomatic development of a single, well-encapsulated subcutaneous mass or nodule at the site of prior trauma. Size varies from 1 to 7 cm diameter, depending in part on the duration of disease. The lesions are normally firm initially, but the center of the nodule may later become necrotic and liquefy, resulting in fluctuance. The overlying skin typically remains intact, unless percutaneous aspiration has been attempted, in which case sinus tracts may form. In one review, 23 of 25 phaeohyphomycotic cysts were on the extremities (11 upper, 12 lower), and two were located on the head, with no lesions found on the trunk.<sup>148</sup>

Fluctuant lesions may be aspirated for diagnostic purposes, revealing tan to brown or gray-green colored contents that are creamy to solid in texture. The cyst fluid can be cultured and examined in 10–20% KOH for the presence of septate, irregularly swollen hyphae that may or may not be branched. Dark yeastlike elements may also be seen, either singly or in chains. These cells can have thickened walls and septa in one plane, but not in more than one plane as seen in the sclerotic bodies of chromoblastomycosis.

Subcutaneous phaeohyphomycosis lesions are often surgically excised, allowing histopathologic examination, which may be needed to differentiate among clinically similar disease processes. Ziefer and Connor<sup>148</sup> described a continuum beginning with solid granulomas (1 of 25 cases) and progressing through multilocular stellate (star-shaped) abscesses (in one-third of cases) to cavitary abscesses (two-thirds) in their series, which corresponds to the three histologic stages (tuberculoid, stellate, and suppurative fluctuant) described earlier by Ichinose.<sup>149</sup> A foreign body such as a splinter fragment will be seen in up to 25% of cases.

The differential diagnosis of subcutaneous phaeohyphomycosis can include fibromas, lipomas, ganglion cysts, chromoblastomycosis, mycetoma, and sporotrichosis. The non-infectious possibilities listed can be readily discounted on examination of tissue specimens, but the other processes are more challenging to exclude.

Culture of the material from aspirated or excised lesions can be plated on media both with and without cycloheximide and chloramphenicol and incubated at 25–30°C. Most of the implicated pathogens will grow within 2 weeks, but plates

should be kept at least 4 weeks before being discarded as negative. The organisms most commonly isolated from these cases include *Exophiala*, *Phialophora*, and *Bipolaris* species.

Surgical excision of the entire lesion is usually curative, and adjunctive antifungal therapy is often not necessary. Lesions that are not amenable to resection are more problematic because incomplete removal of involved tissues invariably results in recurrence. Systemic antifungal therapy is used in such cases, though results are frequently disappointing. The agents that have most frequently been used include amphotericin B, 5-flucytosine, itraconazole,<sup>150</sup> and terbinafine<sup>151</sup>; in vitro synergy testing has been successfully used to guide therapy in an immunocompromised patient with refractory infection.<sup>152</sup> Decisions regarding the duration of medical therapy must be individualized for each case, but several months' treatment is normally required. Documentation of negative histologic findings should be considered before discontinuation of treatment and subsequent close clinical follow-up is recommended.

**Keratitis** Mycotic keratitis, or keratomycosis, is a potentially sight-threatening fungal infection of the cornea. Depending on the study sample, fungi have been found responsible for 6–53% of cases of ulcerative keratitis. Considered as a group, dematiaceous fungi follow *Fusarium* and *Aspergillus* species among the most common etiologic agents; over 20 dematiaceous species from 11 genera have been reported to cause keratitis.<sup>153</sup>

Mycotic keratitis cases have been reported from a worldwide distribution but are more common in tropical and subtropical climates. The incidence of dematiaceous fungal keratitis appears to be seasonal, with the peak in the warm, humid months of late summer and early fall.<sup>154</sup> A large proportion of affected individuals perform agricultural or other outdoor work, and there is a male predominance in mycotic keratitis cases caused by filamentous fungi. Trauma, seemingly minor in many cases, is the main predisposing factor. Plant material such as branches or leaves (or objects or machinery that have been in contact with soil or vegetation) is most frequently implicated. Other mechanisms of trauma, such as surgical manipulation or abrasions caused by contact lenses, could indirectly lead to mycotic keratitis by disrupting the integrity of the corneal epithelium. The use of either antibacterial agents or corticosteroids can predispose to mycotic keratitis or result in a worse outcome in unrecognized cases.<sup>153</sup>

Patients with mycotic keratitis typically seek medical attention between 1 and 21 days of infection, depending on the organism, inoculum, and host immune status. Usually they present with a 5–10-day history of pain, photophobia, lacrimation, and a “foreign body” sensation in the involved eye. Keratitis due to the more common dematiaceous pathogens is normally low grade and slowly progressive over weeks. Infection with *Lasiodiplodia theobromae*, however, typically follows a much more aggressive clinical course.

Despite the above findings, it is difficult to clinically distinguish between bacterial and fungal keratitis. For this reason and because of the potential for development of sight-threatening complications such as endophthalmitis, early diagnosis under the guidance of an ophthalmologist and prompt institution of therapy are essential. The best diagnostic approach is to obtain scrapings from the base and edges of the ulcer using a spatula or surgical blade for direct microscopic examination and culture. If inadequate material is obtained, a corneal biopsy can be sent for histopathology and culture. A direct microscopic

preparation should be made, either with 10–20% KOH or one of several other stains, including Calcofluor white, Giemsa, periodic acid-Schiff (PAS), Gomori methenamine silver (GMS) or lactophenol cotton blue, the relative merits of which are discussed in detail by Thomas.<sup>153</sup> Pigmented fungal elements should be evident in most cases of mycotic keratitis caused by dematiaceous fungi. Culture confirmation is mandatory; a high index of suspicion should be maintained about possible fungal contaminants. Significant isolates are those found on streak lines, and they should preferably be isolated on more than one plate. The most frequently implicated dematiaceous agents of keratitis include *Curvularia*, *Alternaria*, and *Bipolaris* species, and *Lasiodiplodia theobromae*.

Dematiaceous fungal keratitis may respond to antifungal therapy alone when the infection is superficial<sup>155</sup> but surgery, including keratoplasty, is frequently required for deeper infections. Antifungal drugs are most commonly administered topically; because of local toxicity of most agents, subconjunctival injections are rarely used. The polyene drugs natamycin and amphotericin B are the two most frequently used agents. Natamycin 5% solution is considered to be the initial treatment of choice for fungal keratitis. Penetration of natamycin into ocular tissues is poor, however, and it is therefore not recommended for deep ocular fungal infections. Amphotericin B drops (0.1–1%) can be prepared from the intravenous preparation if a commercial preparation is not available. In cases of natamycin treatment failure, amphotericin B has been used either alone or in combination with 5-flucytosine or an azole. Clinical trials investigating the use of combination topical therapy have not been performed.

The kinetics of the azole compounds are advantageous in cases with deeper ocular involvement. Ketoconazole penetrates the cornea well after oral dosing, and combined oral (600 mg/day)/topical (1%) administration has been shown to be effective in treatment of *Curvularia* keratitis. Oral ketoconazole plus topical miconazole has been advocated as a possible first-line treatment for mycotic keratitis. Oral itraconazole is efficacious in treatment of *Aspergillus* keratitis, and there is limited published experience with itraconazole alone or in combination with natamycin for treatment of keratitis caused specifically by the dematiaceous fungi. Fluconazole shows excellent ocular (including corneal) penetration with systemic administration, but it has little activity against the dematiaceous agents implicated in ocular infections. Voriconazole penetrates the eye well after topical and systemic administration<sup>156</sup> and successful treatment of dematiaceous fungal keratitis has been reported.<sup>157</sup> The use of adjunctive topical corticosteroids to prevent inflammation and attendant scarring has been associated with poor outcomes and therefore is not recommended.<sup>153</sup>

**Sinusitis.** Fungal sinusitis cases can be classified into three main categories: allergic fungal sinusitis, fungus ball (also sometimes referred to as sinus “mycetoma,” a usage of the term that should be discouraged), and invasive. Invasive fungal sinusitis can be further subdivided into three categories: acute necrotizing, chronic, and granulomatous.<sup>158</sup> Dematiaceous fungi are common causes of allergic fungal sinusitis, which will be discussed in this chapter, and they may also cause chronic invasive fungal sinusitis and sinus fungus balls.<sup>159</sup>

Allergic fungal sinusitis (AFS) was first reported as a clinical entity in 1981.<sup>160</sup> This process was initially attributed

to *Aspergillus* species because of the similarity of histologic findings to those in allergic bronchopulmonary aspergillosis. Subsequent studies have shown that dematiaceous organisms have grown in more than 80% of culture-positive cases, with the most commonly implicated dematiaceous organisms being *Bipolaris*, *Curvularia*, *Exserohilum*, *Alternaria* and *Cladosporium* species.

Individuals who have allergic fungal sinusitis develop are immunocompetent and typically have a history of chronic sinusitis, with symptom duration ranging from a few months to several years. There is often a history of multiple medical and surgical treatments for chronic sinusitis. Most patients are adolescents or young adults with a history of atopy, nasal polyps and frequently accompanying asthma. Most case series are reported from the southern United States, suggesting that living in a warm, humid climate may be a risk factor. Patients usually present for medical attention when there is an acute worsening of their chronic symptoms. Nasal obstruction and discharge with headache are the most common presenting complaints but more dramatic symptoms, including periorbital swelling, proptosis, and visual disturbances, are also seen. Many patients will report having seen greenish brown concretions in the tissue after vigorous nose blowing.<sup>161</sup>

Allergic fungal sinusitis can be suspected based on imaging results. CT and MRI typically reveal bilateral involvement of multiple sinuses with unilateral predominance and associated bone destruction in 19–64% of cases.<sup>162</sup> Bone destruction does not indicate bone invasion by the fungus; the mechanism in AFS is speculated to be pressure necrosis, the effects of inflammatory mediators, or both. Diagnosis requires operative specimens to be processed appropriately for histologic examination and fungal culture. AFS is characterized by the presence of “allergic mucin” (a mixture of layers of basophilic mucus and sheets of eosinophils with Charcot–Leyden crystals and sparse fungal hyphae) and by the absence of tissue invasion. When black moulds are the cause, use of the Fontana–Masson stain for melanin can increase the sensitivity of histologic examination and reveal the dematiaceous nature of the infecting organism.

Schubert and Goetz<sup>158</sup> have proposed histologic criteria for diagnosis of allergic fungal sinusitis: (1) characteristic allergic mucin seen histopathologically and/or grossly, (2) fungal stain positive in mucin but not in the mucosa, or surgically collected sinus fungal culture is positive in an otherwise characteristic patient, (3) sinus mucosa demonstrates eosinophilic-lymphocytic inflammation without evidence for tissue necrosis, granulomas or fungal invasion, and (4) other fungal diseases are excluded.

Treatment can be challenging, with recurrent disease observed frequently. The most important aspect of management is the surgical removal of the impacted mucin and aeration of the involved sinuses, which can frequently be performed endoscopically. The use of systemic corticosteroids is recommended unless contraindications are present. Schubert suggests prednisone 0.5 mg/kg for the first 2 weeks with a subsequent taper guided by serial total serum IgE determinations, with the goal of maintaining prednisone for 1 year.<sup>158</sup> Systemic antifungal therapy has not been shown definitively to change outcomes, but some physicians have used itraconazole as a component of combined medical/surgical therapy with anecdotal success. Topical intranasal steroid spray and saline irrigations are generally accepted adjunctive measures, and long-term use may

prevent recurrences. Allergen immunotherapy with the aim of reducing the production of fungus-specific IgE production may also prove beneficial.

### Systemic

The processes included in this group are infections that do not have the histologic features of either chromoblastomycosis or mycetoma and that involve deep tissues, thereby excluding them from the other categories of phaeohyphomycosis. Cerebral phaeohyphomycosis is the most frequently reported systemic infection caused by black fungi and will be discussed in greater detail separately below. Less common disease presentations included in this category include infective endocarditis,<sup>163</sup> pulmonary disease,<sup>164</sup> bone and joint infection,<sup>165</sup> dialysis-associated peritonitis,<sup>166</sup> and disseminated disease.<sup>163</sup> Affected individuals are frequently immunocompromised, but both disseminated and localized deep infections have been seen in seemingly immunocompetent individuals.

Because most published reports of systemic disease have been individual case reports, there are relatively few data on which to base treatment recommendations. It appears clear that the best outcomes are achieved with a combined medical and surgical approach when deep tissues are involved, and amphotericin B, itraconazole, and posaconazole would be the agents to consider for the medical component of therapy. Unfortunately, outcomes are poor for patients with disseminated disease in spite of aggressive treatment, with a mortality rate of 79% reported in a review of 72 cases.<sup>163</sup>

**Cerebral phaeohyphomycosis.** Cerebral infections caused by dematiaceous fungi have a worldwide distribution. In a recently published review of 101 cases of primary central nervous system phaeohyphomycosis, the majority of cases were reported from North America (42 cases), Asia (24), the Middle East (15) and Europe (11).<sup>167</sup> There was a male predominance (76%) and although the age range was wide, most individuals were young adults with a mean age of 38 years. Some type of immunocompromising condition was noted for 37% of the patients, but over half (52%) had no identifiable underlying medical conditions.

Most patients in this series presented with symptoms and physical findings compatible with an intracerebral mass lesion. Headache was a frequent complaint (59%), and focal neurologic deficits developed in 54%. Seizures, altered mental status and fever were each observed in approximately one-third of the patients. The most common finding on imaging was brain abscess (87%), 71% of which were single lesions. The second most common presentation was chronic meningitis (9% of cases).

Because of the non-specific clinical features, cerebral phaeohyphomycosis is diagnosed when tissue samples are obtained either at the time of surgery for removal of a mass lesion or postmortem. Pigmented hyphae are most often seen on histologic examination of involved tissues, and the identity of the causative organism is determined by culture. The single most common causative organism is *Cladophialophora bantiana* which was implicated in 48% of the cases reviewed by Revankar and colleagues.<sup>167</sup> *C. bantiana* is clearly neurotropic, as evidenced by central nervous system involvement in 26 of 30 culture-confirmed human infections in one series<sup>168</sup> and confirmed by animal studies.<sup>169</sup> Other dematiaceous fungi that have been implicated include *Ramichloridium mackenziei* (exclusively in the Middle East), *Exophiala dermatitidis*,

*Fonsecaea pedrosoi*, *Curvularia pallescens*, *Ochroconis gallopavum*, *Fonsecaea monophora* and *Bipolaris spicifera*.

Clinical outcomes of cerebral phaeohyphomycosis are almost uniformly poor. Revankar and colleagues reported nearly identical mortality rates for immunocompetent (74%) and immunocompromised (71%) individuals. Survival rates were higher when complete surgical excision of lesions was possible in conjunction with systemic antifungals. All 13 patients who did not receive therapy died, as did 10/13 of those managed with surgery alone; none of the patients infected with *R. mackenziei* survived.

Although the published experience is too small to allow definitive determinations regarding relative efficacies of different antifungal agents, the best outcomes in Revankar's review were in patients who had received amphotericin B in combination with both flucytosine and itraconazole. The newer azoles posaconazole and voriconazole will likely be used increasingly for cerebral phaeohyphomycosis in the future. Case reports have recently been published showing voriconazole to be unsuccessful in treatment of central nervous system *C. bantiana* infection in a small number of immunocompromised patients refractory to other therapies.<sup>170</sup> In spite of this experience, voriconazole merits further study in this clinical situation given its efficacy in some cases of central nervous system aspergillosis. Posaconazole has been shown to improve survival and reduce tissue colony counts in a murine model of cerebral *C. bantiana* infection,<sup>171</sup> and the recent report of successful treatment of a patient with *Ramichloridium mackenziei* infection confirms that it has potential for treating central nervous system infections.

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# Endemic mycoses

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## Introduction

The endemic mycoses are a group of infections caused by dimorphic fungi that exist in a mycelial form in the environment, usually in the soil, but grow as yeasts or spherules at body temperature (Table 15-1). These mycoses include blastomycosis, histoplasmosis, African histoplasmosis, coccidioidomycosis, paracoccidioidomycosis, sporotrichosis, and penicilliosis, and all occur in specific geographic regions and distinct epidemiologic settings. Typically, these infections occur after inhalation of airborne conidia, although in some cases cutaneous inoculation of the fungus occurs.

The clinical manifestations of these infections are highly dependent on the immune status of the host. In an immunocompetent person, primary infection may be minimally symptomatic. However, in patients with compromised host defenses, such as human immunodeficiency virus (HIV) patients and transplant recipients receiving immunosuppressive therapy, primary infection may progress to fulminant disseminated disease. Reactivation of prior disease is also more frequent in this setting, although for some patients the only risk factors for reactivation are advanced age and its accompanying decrease in cell-mediated immunity.<sup>1</sup>

The diagnosis of the endemic mycoses can be challenging when the patient presents after departure from the area of endemicity and now resides in a geographic area where the causative fungus does not exist.<sup>2</sup> In the United States in 2002, there were an estimated 6003 adult and 332 pediatric patients with endemic mycoses (histoplasmosis, coccidioidomycosis, and blastomycosis) that required hospitalization, with crude mortality rates of 5% and 7% among children and adults, respectively.<sup>3</sup> However, these statistics do not reveal the substantial morbidity produced by these diseases, which may require a prolonged duration of treatment, have relapsing courses, and cause permanent disability from pulmonary, osteoarticular, and neurologic involvement.

## Blastomycosis

### Etiology

*Blastomyces dermatitidis* is the etiologic agent of blastomycosis, also called North American blastomycosis. It is the imperfect stage (asexual form) of *Ajellomyces dermatitidis*.

The sexual form is heterothallic and requires opposite mating types for fertile cultures. Infection occurs with equal frequency with both mating types. At 25–30°C, the asexual form initially produces a fluffy white colony on routine mycologic medium. Some strains develop tan, glabrous colonies without conidia, and others produce light brown colonies with concentric rings. On primary isolation, colonies appear in 1–3 weeks. The mould form of *B. dermatitidis* produces 2–10 µm, spherical, ovoid or pyriform conidia located on aerial hyphae and lateral conidiophores. Thick-walled chlamydoconidia 7–18 µm in diameter may be observed in older cultures. The colony and conidia resemble those of *Chrysosporium* spp. and may be indistinguishable from an early culture of *Histoplasma capsulatum*, having only hyphae, conidiophores, and microconidia. In mammalian tissues, broad-based budding yeasts with thick refractile walls varying in diameter from 8 to 30 µm are observed (Fig. 15-1). The yeast form can also be produced in vitro at 37°C on blood agar, inhibitory mould agar or brain-heart infusion (BHI) agar. The colony of the yeast phase is usually cream, with a granular surface.<sup>4-6</sup>

### Epidemiology

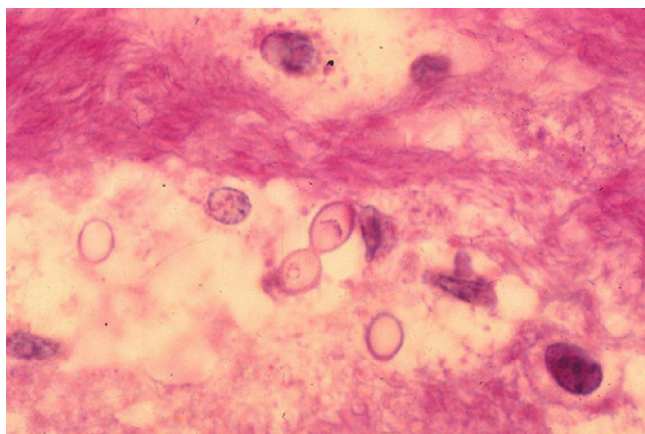
*Blastomyces dermatitidis* commonly occurs along the river estuaries from Minnesota to Mississippi in the United States, the Canadian provinces bordering the Great Lakes, and in scattered areas of Europe, Asia, Latin America, and Africa. Within its endemic area, there are hyperendemic foci; these are areas of warm, moist, sandy, acidic soil, in wooded areas, rich in organic debris, at low elevation in proximity to bodies of water. Although blastomycosis is typically associated with rural areas, urban foci of the disease, also near waterways, have been recently reported.<sup>7</sup> There is some overlap of the endemic areas of blastomycosis and histoplasmosis.<sup>5</sup>

Occupational or recreational soil contact has been associated with outbreaks of infection. Outbreaks may include patients of all ages and both genders, but cases usually occur in young to middle-aged adults and are more commonly reported in men than in women. African-American race and diabetes are risk factors for symptomatic disease.<sup>8,9</sup> Dogs are highly susceptible to blastomycosis and may act as sentinels in point-source outbreaks.<sup>10</sup>

**Table 15-1** Characteristics of systemic dimorphic mycoses

Etiologic agent	Endemic areas	Ecology	Filamentous phase	Yeast phase (Tissue form)	Route of acquisition	Clinical manifestations
<i>Blastomyces dermatitidis</i>	North America (Ohio and Mississippi river valleys) Africa	Decaying organic material	Hyphae, round to oval or pear-shaped, lateral and terminal conidia (2–10 $\mu\text{m}$ in diameter)	Broad-based budding yeasts (8–15 $\mu\text{m}$ in diameter)	Inhalation	Pulmonary disease (<50% of infected individuals) Extrapulmonary: skin, bone, genitourinary tract, CNS (disseminated disease more common in immunosuppressed patients)
<i>Histoplasma capsulatum</i> var. <i>capsulatum</i>  var. <i>duboisii</i>	North America (Ohio and Mississippi river valleys) and Latin America Tropical areas of Africa	Soil with high nitrogen concentration (bird droppings, caves)	Hyphae, large, thick wall spherical to oblong, tuberculate and non tuberculate macroconidia (8–15 $\mu\text{m}$ ) and small, oval microconidia (2–4 $\mu\text{m}$ )	Thin-walled, oval yeasts (2–4 $\mu\text{m}$ ); narrow-based budding  Larger, thick-walled yeasts (8–15 $\mu\text{m}$ ); more prominent isthmus and bud scar	Inhalation	Asymptomatic pulmonary infection (90%) in normal host and low-intensity exposure Disseminated disease more frequent in immunosuppressed and pediatric population Lower rate of pulmonary disease Higher frequency of skin and bone involvement
<i>Coccidioides immitis</i>	Southwestern United States, Mexico, Central and South America	Soil, dust	Hyphae and barrel-shaped arthroconidia (3–6 $\mu\text{m}$ )	Spherules (20–60 $\mu\text{m}$ ) containing endoconidia (2–4 $\mu\text{m}$ )	Inhalation	Asymptomatic pulmonary infection (60%) in normal host Progressive pulmonary infection and dissemination (skin, soft tissues, bone, joints and meninges) more common in immunosuppressed patients
<i>Paracoccidioides brasiliensis</i>	South and Central America	Likely soil associated	Hyphae, oval to globose terminal and lateral microconidia (2–3 $\mu\text{m}$ ) and intercalary chlamydoconidia	Thin to moderately thick-walled, multiply budding yeasts (15–30 $\mu\text{m}$ ) (pilot wheel)	Inhalation	Self-limited pulmonary infection. Organism remains dormant for long period of time, and can cause pulmonary and disseminated disease at a later time if defenses are impaired. Subacute infection in pediatric and immunosuppressed patients with more severe prognosis
<i>Sporothrix schenckii</i>	Scattered worldwide	Soil and thorned plants	Hyphae, small clustered tear-shaped conidia arising from conidiophore and thick-walled brown sessile conidia attached to hyphae	Round or ovoid yeasts (4–6 $\mu\text{m}$ )	Skin inoculation	Lymphangitis subcutaneous lesions Disseminated infection in patients with underlying diseases
<i>Penicillium marneffei</i>	Southeast Asia	Bamboo rats	Hyphae, conidiophores terminating in conspicuous penicillus; ellipsoidal, smooth-walled conidia	Globose to elongated sausage-shaped yeasts (3–5 $\mu\text{m}$ )	Inhalation	Disseminated infection, most commonly in HIV patients. It resembles tuberculosis, leishmaniasis and other AIDS-related opportunistic infections (histoplasmosis, cryptococcosis)





**Figure 15-1** Broad-based singly budding yeasts of *Blastomyces dermatitidis* in tissue section (400×).

## Pathogenesis

The usual route of infection is inhalation of conidia.<sup>6</sup> However, the organism has also been transmitted sexually and by bites from an infected dog.<sup>6,11</sup> In the alveoli, the organism transforms into the yeast and induces an acute inflammatory response that includes neutrophils and macrophages, resulting in granuloma formation. Once in its yeast form, it is relatively resistant to phagocytosis and killing. Cell-mediated immunity, driven by antigen-specific T lymphocytes and lymphokine-activated macrophages, is the principal host defense against the organism and is critical in preventing dissemination. The humoral immune response is not significant in the control of the infection.<sup>6</sup>

The BAD1 cell wall protein (previously known as WI-1), which mediates adhesion, is an indispensable virulence factor for *B. dermatitidis*. BAD1 is expressed only on the yeast forms and is the chief antigenic target for the host immune responses. BAD1 interferes with host immune response by suppressing phagocyte release of tumor necrosis factor (TNF)- $\alpha$ , through transforming growth factor (TGF)- $\beta$  dependent and independent mechanisms.<sup>12</sup> Melanin production has also been recognized as a virulence mechanism of *B. dermatitidis*; this pigment protects the fungus from the reactive oxygen species produced by leukocytes.<sup>13</sup>

## Diagnosis

The diagnosis of blastomycosis requires microscopic demonstration of the fungus in the clinical specimen and confirmation by culture. The most common specimens from patients with suspected blastomycosis are from sputum, bronchoalveolar lavage fluid, and lung biopsies.<sup>5,6</sup> In a study comparing diagnostic methods for blastomycosis, pulmonary cytology was the most successful method for making a diagnosis; culture was negative in one-third of cases.<sup>14</sup> For suppurative cutaneous or visceral lesions, samples should be collected by aspiration. Fresh preparations of sputum, centrifuged cerebrospinal fluid, urine, pus, skin scrapings, and tissue impression smears may be examined directly with Calcofluor white or potassium hydroxide. Tissue and cytologic specimens can be examined with the Grocott-Gomori methenamine silver (GMS), periodic acid-Schiff (PAS), Papanicolaou, and Giemsa stains.<sup>15</sup> The conidia

of the mycelial form, as for *H. capsulatum*, *Coccidioides immitis* and *Paracoccidioides brasiliensis*, are highly infectious when aerosolized. Thus, the plating of specimens and culture should be performed within a biosafety cabinet.<sup>16</sup> Sterile specimens may be inoculated directly onto blood agar, BHI agar, inhibitory mould agar, Sabouraud glucose agar, and enriched broth, such as BHI. Tissues should be minced or homogenized before plating. All cultures should be incubated at 25–30°C under aerobic conditions for 4–8 weeks. The mycelial form of *B. dermatitidis* is non-diagnostic and conversion to the yeast at 37°C is necessary for definitive identification. The identity of the mycelial form can be confirmed by exoantigen testing or a DNA probe.<sup>5</sup>

The currently available serologic tests are not useful for the diagnosis of blastomycosis, because of poor sensitivity and specificity (Table 15-2). Delayed skin hypersensitivity to blastomycin is also unreliable as a diagnostic technique for blastomycosis. A radioimmunoassay technique that utilizes antibodies against the BAD1 surface protein displays high sensitivity and specificity, but is not yet commercially available.<sup>6,17</sup> An antigen detection assay using urine, serum, CSF or BAL fluid has high sensitivity, but there is cross-reactivity with other endemic fungi.<sup>17</sup>

## Clinical findings

Data obtained from blastomycosis outbreaks indicate that although infection rates are high, symptomatic disease occurs in less than one-half of infected individuals. *Blastomyces dermatitidis* causes a wide spectrum of clinical illness, including pulmonary disease and disseminated extrapulmonary disease. The most common extrapulmonary sites of involvement include the skin and soft tissues, bones, genitourinary tract, and central nervous system. Primary cutaneous blastomycosis has occurred after accidental inoculation in the laboratory or at autopsy, and after dog bites.<sup>6,11</sup>

The presentations of pulmonary blastomycosis include:

- asymptomatic, subclinical disease (discovered in outbreak investigations)
- a brief non-specific flu-like illness
- an illness resembling bacterial pneumonia with acute onset, fever, productive cough, and lobar pulmonary infiltrates
- a subacute or chronic respiratory illness resembling tuberculosis or lung cancer and a radiographic presentation of fibronodular infiltrates or mass-like lesions
- a fulminant adult respiratory distress-like syndrome with high fever, diffuse pulmonary infiltrates, and respiratory failure.<sup>6,15,18,19</sup>

The skin is the most frequent site of dissemination, usually presenting as two different types of lesions: verrucous and ulcerative. The more common are the verrucous, which have a raised, irregular border, with crusting and drainage above an abscess in the subcutaneous tissue. The exudative, ulcerative lesions have sharp, heaped-up borders. These lesions can also appear on the mucosa of the nose, mouth, and larynx. The skin lesions of blastomycosis may be misdiagnosed as squamous cell carcinoma, non-tuberculous mycobacterial infections or those of other endemic mycoses (coccidioidomycosis, paracoccidioidomycosis).<sup>6,15</sup>

**Table 15-2** Serodiagnosis of systemic dimorphic mycoses

Mycosis	Test	Comments
Blastomycosis	CF ID EIA RIA (investigational)	Currently available serologic tests: low sensitivity and specificity Detection of specific antibodies against 120 kDa surface protein called WI-1
Histoplasmosis	CF  ID  RIA/ELISA (commercially available: Histoplasma Reference Laboratory, Indianapolis, IN)	Fourfold rise or a titer at least 1:32 suggests active infection. Lower titers also present in 1/3 of active cases. Cross-reactivity with other mycoses Identifies H (more likely in active infections) and M (can be detected in active and chronic infections) precipitin bands. Cross-reactivity with other mycoses Antigen detection: greatest sensitivity in disseminated or acute pulmonary infection. Sensitivity of test greater in urine than in serum. Useful for monitoring of treatment response. Cross-reactivity with other mycoses
Coccidioidomycosis	EIA, ID, TP for IgM detection EIA, ID, CF for IgG detection	Can be detected temporarily in most patients with primary infection  Appears after a few weeks of infection and usually disappears after several months if infection resolves. Elevated titers (>1:16) suggest disseminated, extrapulmonary disease. Combination IDCF commonly used IgG levels in CSF useful in the diagnosis of meningeal disease
Paracoccidioidomycosis	ID CF	Specific, but not useful for monitoring response Useful for monitoring response. Cross-reactivity with other mycoses
Sporothricosis	LA	Not widely used
Penicilliosis	Under development	

Key: CF, complement fixation; ID, immunodiffusion; EIA, enzyme immunoassay; RIA, radioimmunoassay; ELISA, enzyme-linked immunosorbent assay; TB, tube precipitin; LA, latex agglutination.

Other extrapulmonary sites of infection include the bones (particularly vertebrae, skull, ribs, and long bones), genitourinary tract (prostate, epididymis, kidney), and central nervous system (51% of cases, manifesting as meningitis, and epidural or cranial abscesses). Blastomycosis may infrequently involve the liver, spleen, gastrointestinal tract, thyroid, pericardium, and adrenal glands.<sup>6</sup>

Patients with underlying defects in T cell function (such as HIV infection, glucocorticoid use, hematologic malignancy, and solid organ transplantation) are predisposed to severe, relapsing disease, which is associated with a high mortality rate during the first few weeks after the onset of symptoms. In this population, pulmonary disease is more likely to present with diffuse pulmonary infiltrates and respiratory failure. Dissemination to multiple organs, including the CNS, also occurs more frequently.<sup>20</sup>

## Treatment

Practice guidelines for the management of blastomycosis were published in 2008.<sup>21</sup> The decision to treat patients with blastomycosis involves consideration of the clinical form and severity of the disease, the immunocompetence of the patient, and the toxicity of the antifungal agent. There are no randomized, controlled trials, so the recommendations come from case series and expert opinion. For pulmonary blastomycosis, all immunocompromised patients and patients with progressive pulmonary infection should be treated (Table 15-3). Although controversial, in a few selected cases of acute pulmonary blastomycosis, therapy may be withheld and the patient should be followed for years for evidence of reactivation or progression. Patients with life-threatening disease, such as acute respiratory distress syndrome, should be treated with amphotericin B (0.7–1 mg/kg/day; total dose 1.5–2.5 g). Lipid formulations of amphotericin B have been less extensively studied in blastomycosis but are less toxic than amphotericin B deoxycholate and are recommended in patients with severe infection. Use should be restricted to patients who cannot tolerate conventional amphotericin B. Therapy for some patients may be switched to itraconazole (200–400 mg/day) after clinical stabilization in seriously ill patients with an initial course of amphotericin B treatment, usually a minimum cumulative amphotericin B dose of 500 mg. Patients with mild to moderate disease should be treated with itraconazole at 200–400 mg/day for a minimum of 6 months. An alternative to itraconazole includes 6 months of either ketoconazole at 400–800 mg/day or fluconazole at a dosage of 400–800 mg/day. However, except for CNS disease, fluconazole is considered to be less effective than itraconazole in the treatment of blastomycosis. For patients who are unable to tolerate an azole or whose diseases progress during azole treatment, therapy should be changed to amphotericin B (0.5–0.7 mg/kg/day; total dose, 1.5–2.5 g).

All patients with severe disseminated disease require treatment. Patients with CNS infection should receive a dosage of amphotericin B of 0.7–1 mg/kg/day (total dose, at least 2 g). Patients with life-threatening disease should be treated with amphotericin B (0.7–1 mg/kg/day; total dose, 1.5–2.5 g). Therapy for some patients may be switched to itraconazole after clinical stabilization with amphotericin B. Patients with mild to moderate disseminated blastomycosis that does not involve the CNS should be treated with itraconazole (200–400 mg/day) for at least 6 months. Ketoconazole and fluconazole, both at dosages of 400–800 mg/day, are alternatives to itraconazole.<sup>21</sup>

Osseous involvement in blastomycosis is difficult to treat and is prone to relapse. Thus, blastomycotic osteomyelitis should be treated with an azole for at least 1 year. For patients who are intolerant of azoles or fail azole treatment, amphotericin B (0.5–0.7 mg/kg/day; total dose, 1.5–2.5 g) is recommended. In conjunction with antifungal therapy, surgery is indicated for the drainage of large abscesses, for sizeable collections of empyema fluid or bronchopleural fistula, and for the debridement of devitalized bone in patients with osteomyelitis who are responding poorly to therapy.<sup>21</sup>

For immunocompromised patients, because of their tendency to develop rapidly progressive disease with worse prognosis, early and aggressive treatment with amphotericin B (0.7–1 mg/kg/day; total dose 1.5–2.5 g) is recommended. In selected patients without CNS infection, treatment can be switched to itraconazole after initial stabilization with amphotericin B (usually a minimum dose of 1 g). Because of the high rate of relapses in this immunosuppressed population, chronic suppressive therapy with an azole, preferably itraconazole, is recommended. Ketoconazole is not recommended because relapse rates are higher. Fluconazole, at 800 mg or higher per day, is an option for those patients with CNS disease or intolerance to itraconazole.<sup>21</sup>

For pregnant women, amphotericin B (1.5–2.5 g) is preferred because of the teratogenicity of azoles. Progressive, severe or disseminated disease in children should be treated with amphotericin B (total dose,  $\geq 30$  mg/kg). A limited number of pediatric patients with non-life threatening disease have been successfully treated with itraconazole (5–7 mg/kg/day).<sup>21</sup>

The extended-spectrum azole has in vitro activity against *Blastomyces* but there are limited data to support their clinical use. Voriconazole has been successfully used to treat cerebral blastomycosis in a small case series.<sup>22</sup> There is no published clinical experience with posaconazole or the echinocandins.

In a recent series from Canada,<sup>23</sup> the death rate in patients with blastomycosis was 6.3% but in a study from Missouri,<sup>8</sup> the mortality rate was 44%. The death rate is strongly influenced by factors that delay diagnosis, such as the initial lack of recognition of the disease in areas of lower incidence.<sup>8</sup> In the endemic area, clinicians should consider blastomycosis in the differential diagnosis of lung, skin, bone, and genitourinary diseases. Advances in molecular diagnostic tests have the potential for improving case detection of blastomycosis and decreasing the delay in diagnosis.<sup>24</sup>

## Histoplasmosis

### Etiology

Human histoplasmosis is caused by two varieties of *Histoplasma capsulatum*: *H. capsulatum* var. *capsulatum* and *H. capsulatum* var. *duboisii*. *H. capsulatum*, like *B. dermatitidis*, produces an ascomycetous teleomorph, *Ajellomyces capsulatus*, when two compatible isolates are paired on agar media such as soil extract agar. At 25–30°C, the asexual form produces moderately growing, expanding, granular to cottony, initially white colonies that later become brownish. On primary isolation, small hyphal colonies appear after several days to a week. The mould or saprophytic form of *H. capsulatum* produces two types of conidia: large (8–15  $\mu$ m)



**Table 15-3** Antifungal treatment for systemic dimorphic mycoses

<b>Mycosis</b>	<b>First choice</b>	<b>Alternative treatment</b>
<b>Blastomycosis</b>		
Life-threatening or meningeal	L-AmB or AmB	
Mild or moderate	Itra	Flu, Vori, Posa, Keto
<b>Histoplasmosis</b>		
Severe or CNS	L-AmB	AmB as alternative therapy for Server or CNS
Mild	Itra	Posa, Vori, Flu
<b>Coccidioidomycosis</b>		
Diffuse pneumonia	AmB	Itra, Posa, Vori
Extrapulmonary, non-meningeal	Itra, Flu	Posa, Vori
Severe, meningeal	Flu, Itra	Posa, Vori
Mild	Itra	Flu
<b>Paracoccidioidomycosis</b>		
Juvenile and adult form	Itra	
Severe or refractory	L-AmB or AmB followed by sulfonamide or azole	Keto
<b>Sporothricosis</b>		
Lymphocutaneous	Itra	SSKI, terbinafine, local hyperthermia
Disseminated	AmB	
<b>Penicilliosis</b>	AmB	Itra
Key: AmB, amphotericin B; L-AmB, lipid formulation of Amphotericin B; Itra, itraconazole; Flu, fluconazole; Keto, ketoconazole; SSKI, saturated solution of potassium iodide.		

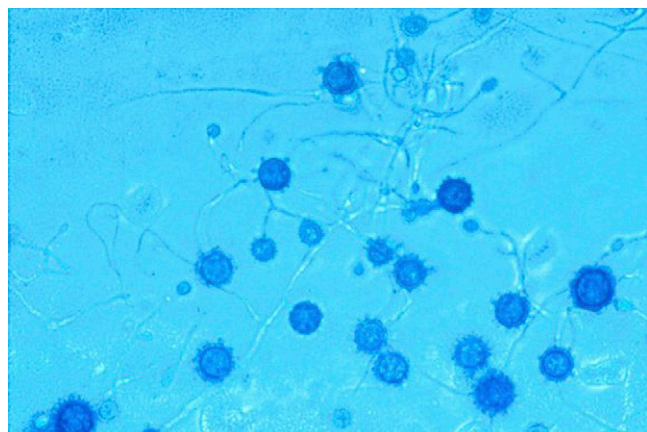
thick-walled, spherical or oblong to pyriform macroconidia with finger-like projections (tuberculate conidia) that arise from short conidiophores, and small, oval microconidia (2–4  $\mu\text{m}$ ) with smooth or roughened walls that are sessile or arise on short stalks from undifferentiated hyphae (Fig. 15-2). The yeast form appears in mammalian tissues or when conidia are inoculated at 37°C in BHI with blood agar. The colonies of the yeast form are smooth or wrinkled but never mucoid. The color is initially cream to beige, turning to gray as the colony ages. The yeast cells are oval with thin walls and measure 2–4  $\mu\text{m}$ . They multiply by polar budding, and the isthmus between the mother and the daughter cells is narrow.

The yeast cells of *H. capsulatum* are difficult to differentiate from a rare small form of *B. dermatitidis*. The yeast cells of *H. capsulatum* are intracellular and uninucleate, whereas those of *B. dermatitidis* are multinucleate and do not grow intracellularly. The budding cells of var. *duboisii* in tissue differ from those of var. *capsulatum* in their larger size (8–15  $\mu\text{m}$ ), thicker walls, and more prominent bud scar or isthmus.<sup>5,25-27</sup>

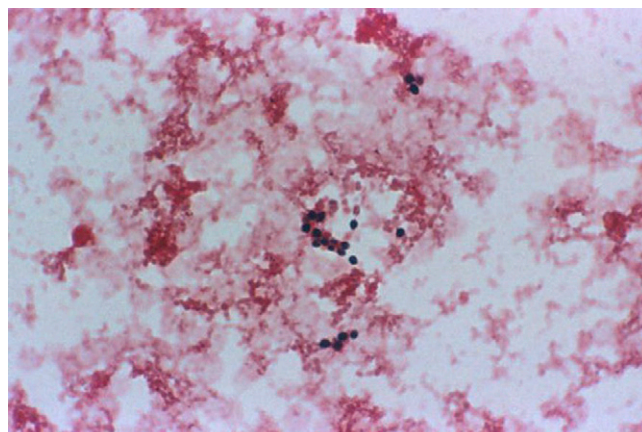
## Epidemiology

The most highly endemic areas for *H. capsulatum* var. *capsulatum* include the eastern United States (Ohio, Mississippi, and St. Lawrence River valleys) and most of Latin America. The natural habitat of the mycelial form of *H. capsulatum* is soil with a high nitrogen content, such as areas contaminated with bird and bat guano, and is associated with bird roosts, caves, and dilapidated buildings.<sup>25</sup> There have been several outbreaks associated with exposure to these reservoirs. In a study of HIV-infected persons with histoplasmosis, those with occupational exposure to soil contaminated with bat or bird droppings had a 3.3-times greater risk of acquiring the infection.<sup>28</sup> The attack rate approaches 100% in certain endemic areas but most cases remain asymptomatic and are detected only by skin testing.

Immunocompromised patients and children are more prone to develop symptoms after primary infection. Reactivation of the disease is also common in immunosuppressed populations, such as those receiving chemotherapy and



**Figure 15-2** *Histoplasma capsulatum* mould phase macroconidia (cotton-blue preparation, 400×).



**Figure 15-3** *Histoplasma capsulatum* yeasts on peripheral blood smear (400×).

patients with AIDS.<sup>29</sup> Persons receiving TNF antagonists, children with cancer, and pregnant women represent newly recognized susceptible hosts for histoplasmosis.<sup>30-32</sup> It has also been increasingly diagnosed in travelers to Latin America, Africa, and the Caribbean, especially those who engage in spelunking.<sup>33</sup>

African histoplasmosis, caused by var. *duboisii*, is found in Central and Western Africa between the latitudes 15°N and 10°S, roughly between the Sahara and Kalahari deserts; the majority of cases have been reported from Nigeria. The ecology of the var. *duboisii* is not well known, but it has been found in bat caves<sup>34</sup> (see Table 15-1).

## Pathogenesis

Microconidia of *H. capsulatum* (and, to a lesser degree, macroconidia and small hyphal elements) are inhaled into the lungs, where they reach the alveolar spaces and transform into yeasts. Neutrophils and alveolar macrophages phagocytose the organisms and exert their antifungal activity through poorly understood killing mechanisms which do not involve respiratory burst.<sup>35</sup> Macrophages display less efficient antifungal activity and yeast forms multiply within them, leading to lysis and the infection of new cells.<sup>36</sup> T helper (CD4) cells are crucial to resolving the infection, whereas cytotoxic (CD8) cells have an additive effect to the CD4 lymphocytes for optimal eradication of the organisms. This effect has been demonstrated in mice and observed in patients with CD4 deficiency, such as those with AIDS.<sup>37-39</sup>

Following pulmonary infection, organisms spread through lymphatics to the regional lymph nodes and hematogenously to other organs. In immunocompetent persons, the pathologic findings typically resemble tuberculosis, with granulomas occasionally evolving to caseous necrosis. These granulomas heal with fibrosis and may calcify. An immunologic reaction to the organism or antigens released from killed organisms is likely responsible for arthritis, pericarditis, and erythema nodosum associated with the primary infection. Patients with AIDS show a minimal inflammatory response, and masses of organisms are commonly observed.<sup>40,41</sup>

## Diagnosis

Definitive diagnosis of histoplasmosis requires growth of the fungus from samples of body fluids or tissues. The diagnosis of histoplasmosis can be rapidly established by observation of the yeast phase by direct microscopic examination of specimens with special stains. Because of the small size of the yeast form, it can be confused with *Candida glabrata*. The Wright–Giemsa stain allows detection of the intracellular yeasts of *H. capsulatum* in sputum, blood smears, bone marrow aspirates, and biopsy specimens (Fig. 15-3). The yeast phase of *H. capsulatum* in vivo must also be distinguished from the intracellular parasites *Leishmania donovani* and *Toxoplasma gondii*, small forms of *B. dermatitidis*, the yeast cells of *Penicillium marneffei* and *Cryptococcus neoformans*, and the endoconidia and young spherules of *Coccidioides immitis*. Suspected cultures should be manipulated only in a biologic safety cabinet (class III agent) owing to the risk of infection from inhaled aerosols.<sup>16</sup>

Diagnosis by cultures is limited by a low rate of positivity in self-limited disease and the slow growth of the organism (up to 6 weeks).<sup>25</sup> More rapid growth of cultures is observed in patients with a heavy organism burden, such as patients with AIDS. More than 75% of patients with disseminated disease have positive blood, bone marrow or urine cultures. The most effective method to recover the fungus from blood is the lysis centrifugation technique. The mycelial form of *H. capsulatum* is not diagnostic and conversion to the yeast form at 37°C is necessary for definitive identification. The identity of the mycelial form can also be confirmed using the exoantigen testing or, preferably, a DNA probe.<sup>5,25</sup> Cultures are often positive in patients with disseminated infection, chronic cavitary pulmonary disease, and in more severe acute pulmonary histoplasmosis, whereas cultures are usually negative in moderate acute pulmonary histoplasmosis, granulomatous mediastinitis, mediastinal fibrosis, and meningitis.<sup>25</sup>

Skin tests for histoplasmosis are not useful to establish a diagnosis because most patients in endemic zones have skin test reactivity to histoplasmin that is retained for years after primary infection. Patients with disseminated histoplasmosis may have a negative skin test reaction. Detection of antibodies

to yeast- and mycelial-phase antigens is used to diagnose acute infection, although cross-reaction with other fungi can occur (see Table 15-2). Antibodies usually appear 4–8 weeks after acute symptomatic infection and gradually decrease over a 2–5-year period. A fourfold rise in complement fixation (CF) titers or a titer of at least 1:32 suggests active infection. In chronic pulmonary histoplasmosis, titers are usually lower. Although weakly positive titers are less helpful in differentiating active from past infection, they should not be disregarded because titers in this range occur in about one-third of cases with active disease. The immunodiffusion (ID) assay measures H and M band antibodies. Whereas the H band is more specific for the diagnosis, the M band is more sensitive. The diagnostic yield is enhanced by using both ID and CF tests.

A radioimmunoassay and an enzyme immunoassay have been developed for detecting *Histoplasma* antigen in serum and urine. These methods are particularly sensitive in disseminated and diffuse pulmonary infection (90% and 80%, respectively). The sensitivity of antigen detection is greater in urine samples compared to serum. Serial measurement of antigen provides a means for assessing efficacy of therapy and for establishing relapse. However, cross-reaction of the antigen test can occur with patients infected with other mycoses (paracoccidioidomycosis, blastomycosis, penicilliosis).<sup>17,42-47</sup>

## Clinical findings

### *Histoplasma capsulatum* var. *capsulatum*

A comprehensive review of the clinical aspects of histoplasmosis has recently been published.<sup>25</sup> The clinical spectrum of histoplasmosis depends on underlying host immune status, the intensity of exposure, and prior immunity. After low inoculum exposures, asymptomatic infection occurs in 90% of the patients. However, after a heavy exposure, most infections are symptomatic. Acute self-limited pulmonary histoplasmosis is characterized by a flu-like illness with fever, chills, headache, myalgias, anorexia, non-productive cough, and chest pain. Chest radiographs may show enlarged hilar or mediastinal lymph nodes with patchy infiltrates. After a heavy exposure, more extensive pulmonary disease may occur, including adult respiratory distress syndrome. Rheumatologic complications may accompany acute symptomatic infection in approximately 10% of patients and include arthritis or arthralgias accompanied by erythema nodosum. Pericarditis may also develop in up to 5% with acute infection. The differential diagnosis of localized *Histoplasma* pneumonia includes infection with *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Mycobacterium tuberculosis*, *B. dermatitidis* and *C. immitis*.

Acute pulmonary histoplasmosis usually resolves without therapy but residual calcified granulomas are commonly observed on chest radiographs of persons residing in the endemic areas. In about 1 per 100,000 incident cases, progressive pulmonary histoplasmosis follows acute infection or from reactivation of prior disease. This condition is characterized by chronic pulmonary symptoms associated with apical pulmonary cavitation and fibrosis. This entity is more likely to develop in immunocompromised patients and those with preexisting lung disease. Less than one-third of the lesions heal spontaneously. More commonly, persistence of the organism will lead to chronic infection and fibrosis, with progressive infection occurring in over 50% of patients. In approximately

1 in 2000 adults, progressive disseminated histoplasmosis (PDH) follows acute infection. This condition is much more common among pediatric patients and immunocompromised adults.

The clinical spectrum of PDH includes chronic, subacute, and acute syndromes. Chronic PDH is characterized by gradual weight loss, fatigue, and, in one-third of the patients, fever. The most common findings are oropharyngeal lesions and hepatosplenomegaly. Subacute PDH is characterized by fever, weight loss, and malaise. Oropharyngeal ulcers may be present and hepatosplenomegaly is a common feature. Anemia, leukopenia, and thrombocytopenia are present in one-third of the patients. Other common sites of involvement are the adrenals, aortic and mitral valves, and the central nervous system. If untreated, subacute PDH progresses to death within 2–24 months. Acute PDH is more likely to occur in immunocompromised patients, infants, and those with debilitating medical conditions. Fever, pulmonary infiltrates (including adult respiratory distress syndrome), and septic shock may occur. Dissemination can result in gastrointestinal and oropharyngeal ulceration and bleeding, skin lesions, adrenal insufficiency, meningitis, and endocarditis. Signs and symptoms of disseminated infection are non-specific and can include fever, weight loss, hepatosplenomegaly, and lymphadenopathy. Chest radiographs reveal a diffuse interstitial or reticulonodular infiltrate. The most common laboratory abnormalities include elevated hepatic enzymes and cytopenias. If untreated, acute PDH is usually fatal within weeks.<sup>25</sup>

### *Histoplasma capsulatum* var. *duboisii*

African histoplasmosis is characterized by skin, subcutaneous, and bone disease. Chronic ulcers and subcutaneous nodules are common. Bone infection manifests as multiple osteolytic lesions that may form chronic draining sinus tracts.<sup>48</sup> There is no clear correlation between compromised host immune status and infection with var. *duboisii*.

## Treatment

Practice guidelines for the treatment of histoplasmosis were published in 2007.<sup>49</sup> The first decision that should be made in the management of histoplasmosis is whether or not to treat with antifungals, since most patients recover without therapy. For asymptomatic pulmonary infection, no specific treatment is required. For immunocompetent patients with severe acute pulmonary histoplasmosis, lipid formulation of amphotericin B (3.0–5.0 mg/kg daily intravenously for 1–2 weeks) followed by itraconazole (200 mg 3 times daily for 3 days, and then 200 mg twice daily for a total of 12 weeks) is recommended. The deoxycholate formulation of amphotericin B (0.7–1.0 mg/kg daily intravenously) is an alternative to a lipid formulation in patients who are at a low risk for nephrotoxicity. Prednisone (0.5–1.0 mg/kg/day for 1–2 weeks) is recommended as initial therapy in patients with respiratory compromise as initial therapy. Response rates with these regimens in HIV-infected and non-HIV-infected-patients is more than 75%. A rapid response (less than 1 week) has been observed.<sup>50</sup> In mild or moderately severe acute disease, itraconazole is the drug of choice, at a dose of 400 mg/day initially, then 200 mg/day for a total of 6–12 weeks in non-AIDS patients. For immunocompetent patients with disseminated disease, a similar therapeutic



approach should be applied but the duration of treatment should be extended to 6–18 months. For chronic pulmonary histoplasmosis, itraconazole (200–400 mg/day) should be given for 12–24 months. In AIDS patients, prolonged suppression with itraconazole should be used.<sup>51</sup> However, suppressive therapy can be discontinued in AIDS patients with histoplasmosis who achieve immune reconstitution to a CD4 count of 200 cells/ $\mu$ l for 1 year on antiretroviral therapy.<sup>52</sup>

Fluconazole is also effective in the treatment of histoplasmosis, but it is less potent than itraconazole. High-level fluconazole resistance and the development of fluconazole resistance while on therapy has been documented with *H. capsulatum*.<sup>53</sup> For *H. capsulatum* meningitis, liposomal amphotericin B (5.0 mg/kg day for a total of 175 mg/kg given over 4–6 weeks) followed by itraconazole (200 mg 2 or 3 times daily) for at least 1 year and until resolution of CSF abnormalities, including *Histoplasma* antigen levels, is recommended.<sup>49</sup>

Blood cultures and antigen assays revert to negative more rapidly in patients given liposomal amphotericin than itraconazole.<sup>54</sup> In a study of HIV-infected patients, liposomal amphotericin B was found to be less toxic and more effective than conventional amphotericin B.<sup>55</sup> Posaconazole and voriconazole have been used successfully in salvage therapy of patients with histoplasmosis who have failed treatment with amphotericin B and itraconazole.<sup>56</sup> In lymphocyte-depleted mice, posaconazole was as effective as amphotericin B and more effective than itraconazole.<sup>57</sup> However, caution is warranted when trying to salvage patients with fluconazole failure with voriconazole, because isolates with decreased fluconazole susceptibility may also have decreased susceptibility to voriconazole.<sup>58</sup>

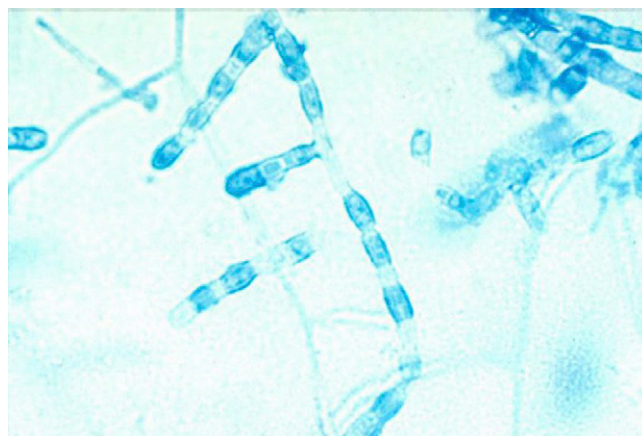
In non-AIDS cases of histoplasmosis with adult respiratory distress syndrome, steroids may be used as adjunctive therapy.<sup>59</sup> However, steroids have no role in the management of histoplasmosis in AIDS, because the infiltrates are composed primarily of massive numbers of organisms rather than host inflammatory cells. Rheumatologic manifestations respond to antiinflammatory drugs without antifungal treatment.

The prognosis is excellent for acute pulmonary disease in the immunocompetent patient. In contrast, fibrosing mediastinitis is usually slowly progressive and often fatal. Acute disseminated disease is also usually fatal if untreated.<sup>25</sup> In studies of AIDS patients with disseminated histoplasmosis, death rates may be as high as 32%. Factors associated with fatal outcome include hemoglobin <8.0 g/dl, AST level >2.5 times the upper limit of normal, acute renal failure, respiratory insufficiency, thrombocytopenia, and LDH level twice the upper limit of the normal range.<sup>60,61</sup>

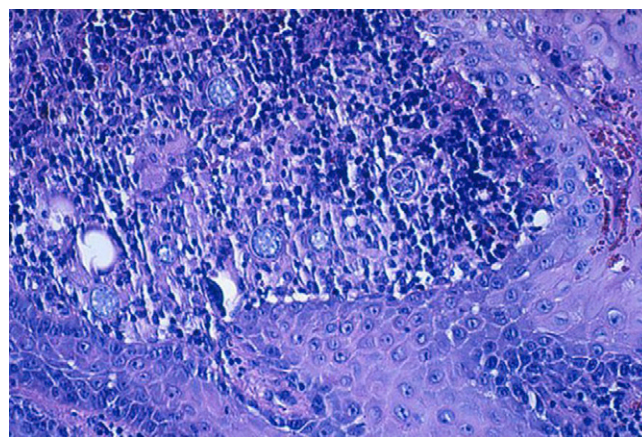
## Coccidioidomycosis<sup>62,63</sup>

### Etiology

Coccidioidomycosis is caused by soil fungi of the genus *Coccidioides*, recently divided on the basis of genomics into *C. immitis* (primarily California isolates) and *C. posadasii* (isolates primarily outside California).<sup>64</sup> With no clear clinical differences, both *C. immitis* and *C. posadasii* will be herein referred to as *C. immitis*. The organism is endemic to the southwestern United States, northern Mexico, and scattered areas of Central and South America. At 25–30°C the fungus grows as a mould



**Figure 15-4** Arthroconidia of *Coccidioides immitis* with typical alternating “ghost cells” (cotton-blue preparation, 400 $\times$ ).



**Figure 15-5** *Coccidioides immitis* spherules filled with endoconidia in a tissue biopsy (arrow).

and colonies are usually white but may be tan to brown, pink, purple or yellow. The hyphae are thin, septate, and hyaline. Thicker side branches give rise to unicellular, barrel-shaped arthroconidia (3–4  $\times$  3–6  $\mu$ m) alternating with empty disjunctive cells (Fig. 15-4). At 37°C, in tissue or special media, the arthroconidia become spherical, enlarge and develop into spherules (20–60  $\mu$ m) that contain endoconidia (2–4  $\mu$ m) (Fig. 15-5).<sup>5,65</sup>

### Epidemiology

*Coccidioides* occurs naturally only in the Western hemisphere, primarily in the southwestern United States and Mexico. The endemic areas coincide with the Lower Sonoran Life Zone, characterized by arid to semi-arid climates, hot summers, low altitude, alkaline soil, and sparse flora. Hyperendemic areas include Kern, Tulare, and Fresno counties in the San Joaquin Valley of California and Pima, Pinal, and Maricopa counties in Arizona. Major cities within these hyperendemic areas include Bakersfield, CA, and Phoenix and Tucson, AZ.<sup>66</sup> *Coccidioides* is also endemic to parts of Central America (Guatemala, Honduras, and Nicaragua), and South America (Argentina, Paraguay, Venezuela, and Colombia) (see Table 15-1). In the United States, an estimated 150,000 cases of

coccidioidomycosis occur annually, with clinical manifestations ranging from a self-limited upper respiratory infection to disseminated disease.<sup>67</sup>

Cycles of rain and drought enhance dispersion of the organism because rains facilitate growth of the organism in the soil and subsequent drought conditions favor aerosolization of arthroconidia.<sup>68</sup> An increase in the number of cases was seen in the 1980s and 1990s because of the incidence of infection in patients with AIDS and an epidemic of coccidioidomycosis in California.<sup>29,69</sup> Factors responsible for the California epidemic were: a drought–rain cycle; new construction, resulting in soil disruption; and an influx of susceptible individuals into the area.<sup>69</sup> Outbreaks of coccidioidomycosis may follow natural events that result in atmospheric soil dispersion, such as dust storms, earthquakes, and droughts; notable events include a 1977 dust storm in the San Joaquin Valley and the Northridge earthquake (California) in 1994.<sup>70,71</sup> Persons with occupations which include soil exposure, such as agricultural workers, excavators, military personnel, and archeologists, are at the greatest risk of acquiring coccidioidomycosis.<sup>72,73</sup> Immunocompromised persons are also at high risk, including patients with AIDS,<sup>74</sup> transplant recipients (especially those who receive *Coccidioides*-infected organs),<sup>75</sup> patients treated with TNF- $\alpha$  antagonists,<sup>76</sup> pregnant women, especially in the third trimester,<sup>77</sup> and cancer patients.<sup>78</sup> Certain ethnic groups and persons with blood group B are also at high risk to suffer disseminated disease.<sup>79</sup> Persons of Filipino or African-American descent have a 10–175-fold higher risk for dissemination.<sup>72</sup>

## Pathogenesis<sup>80</sup>

The usual portal of entry for *Coccidioides* is inhalation of arthroconidia. A single arthroconidium may be sufficient to produce a naturally acquired respiratory infection. Pulmonary macrophages and neutrophils provide the initial host defense. Arthroconidia germinate to produce spherules filled with endoconidia, which is the characteristic tissue phase of the organism. Spherules rupture to release endoconidia, which form additional spherules. The spherules become surrounded by neutrophils and macrophages, which leads to granuloma formation. Arthroconidia, endoconidia, and spherules are resistant to killing by these cells, which may be related to components of the outer wall of the organism.<sup>62,80</sup> Recently described virulence factors of *Coccidioides posadasii* include melanin and extracellular urease.<sup>81,82</sup> The urease produces an alkaline microenvironment which impairs granuloma formation and pathogen clearance.<sup>82</sup> *Coccidioides* infection of the lung also alters pulmonary surfactant production, which may also promote disease progression.<sup>83</sup> Cell-mediated defenses with T lymphocytes are central to the immune response. Disseminated infection occurs in immunocompromised patients with deficient cell-mediated in certain ethnic groups for unclear reasons.<sup>72,80</sup>

## Diagnosis

It is preferable to establish the diagnosis of coccidioidomycosis by direct demonstration of the organism by histologic staining of tissue or wet mounts of sputa or exudates processed with KOH or Calcofluor, because laboratory personnel are at risk of disease acquisition by handling cultures. The observation

of spherules with endoconidia in specimens is pathognomonic. The differential microscopic diagnosis of endoconidia and small spherules includes atypical forms of *B. dermatitidis* and non-budding yeasts of *H. capsulatum*, *P. brasiliensis*, *C. glabrata*, and *C. neoformans*. In addition, the alga *Prototheca wickerhamii* resembles small spherules and the protozoan *Rhinosporidium seeberi* may be confused with larger spherules. *Coccidioides*, unlike *H. capsulatum* and *B. dermatitidis*, has a rapid growth rate. The growth will be evident in 3–5 days and sporulation may be seen after 5–10 days.<sup>5</sup> The same biosafety measures must be employed as described for *H. capsulatum*.<sup>16</sup> Conversion to spherules is not a routine procedure and definitive identification can be made by DNA probe or exoantigen testing.<sup>5</sup>

Serologic methods are particularly useful in establishing a diagnosis of coccidioidomycosis and following the course of disease (see Table 15-2). Serologic tests may be positive in 90% of cases of coccidioidomycosis; however, the sensitivity may be lower in patients with AIDS and antibodies may be undetectable during the first 3 months following acute infection.<sup>17</sup> IgM antibodies may be present soon after infection or relapse but then wane; quantification does not correlate with disease severity. The IgM antibodies can be detected by a tube precipitin test, in which the serum is combined with a soluble antigen to form a precipitate. If the test is performed as a diffusion assay using agar, it is termed the immunodiffusion tube precipitin (IDTP) test.<sup>62</sup>

The anticoccidial IgG antibody appears later than the IgM antibody and remains positive for months. The IgG antibodies are able to fix complement when combined with coccidioidal antigen, and can be detected by immunodiffusion techniques (IDCF). Rising titers of IgG are associated with progressive disease, while declining titers are associated with resolution. Patients with immunodiffusion complement fixation (IDCF) titers of  $\geq 1:16$  are more likely to have disseminated disease. In the CSF, a positive IDCF of any titer is considered diagnostic of meningitis and serology is much more sensitive than culture in making the diagnosis.<sup>17</sup> An FDA-approved EIA is available but its sensitivity is no better than ID and it is less specific. Also, the results correlate poorly with CF titers and do not reflect prognosis.<sup>84</sup> No specific antigen test is available for coccidioidomycosis; however, for patients with acute disease, there is often a false-positive result for the *Histoplasma* urine antigen test.<sup>85</sup> PCR methods for the rapid diagnosis of coccidioidomycosis have been developed but are not available clinically.<sup>86</sup>

## Clinical findings<sup>62</sup>

Coccidioidomycosis is asymptomatic in 60% of infected individuals and infection is detected only by a positive skin test. In the remaining 40%, a self-limited, flu-like illness, with dry cough, pleuritic chest pain, myalgias, arthralgia, fever, sweats, anorexia, and weakness, develops 1–3 weeks after exposure. Primary infection may be accompanied by a variety of immune complex-mediated complications, including an erythematous macular rash, erythema multiforme, and erythema nodosum. Acute infection usually resolves without therapy, although symptoms may persist for weeks. In 5% of these patients, asymptomatic pulmonary residua persist, including pulmonary nodules and cavitation. Immunocompromised patients



**Figure 15-6** Magnetic imaging scan showing destructive spine lesion typical of coccidioidomycosis.

are more susceptible to developing chronic progressive pulmonary infection characterized by the presence of extensive thin-walled cavities that may be complicated by cavity rupture, with bronchopleural fistula and empyema formation.

Symptomatic extrapulmonary disease develops in 1 in 200 patients and can involve the skin, soft tissues, bones, joints, and meninges. The most common cutaneous lesions are verrucous papules or plaques. The spine is the most frequent site of osseous dissemination (Fig. 15-6), although the typical lytic lesions may also occur in the skull, hands, feet and tibia. Joint involvement is usually monoarticular and most commonly involves the ankle and knee. *Coccidioides* fungemia, often fatal, has been recently described in immunocompromised patients.<sup>87</sup> In coccidioidal meningitis, the basilar meninges are usually affected. Cerebrospinal fluid (CSF) findings include mononuclear pleocytosis (often with eosinophilia), hypoglycorrachia, and elevated protein levels. The mortality is greater than 90% at 1 year without therapy, and chronic infection is common. The presence of hydrocephalus or hydrocephalus coexisting with infarction by neuroimaging studies is associated with a higher mortality rate.<sup>88</sup>

## Treatment

Practice guidelines for the management of coccidioidomycosis were published in 2005.<sup>67</sup> In most patients, primary pulmonary infection resolves spontaneously without specific antifungal therapy. For patients with concurrent risk factors, such as HIV infection, organ transplant or corticosteroid therapy, or those with unusually severe infection, treatment is necessary (see Table 15-3). Also, primary infection occurring during the

third trimester of pregnancy or immediately in the postpartum period requires therapy.

Patients with diffuse pneumonia, which is more common in the immunosuppressed population, therapy with amphotericin B, 0.5–0.7 mg/kg/day, should be started and maintained for several weeks, followed by oral azole antifungal therapy (itraconazole, 200 mg twice a day, or fluconazole, 400–800 mg per day). The total duration of therapy should be at least 1 year, and in immunosuppressed patients, oral azole therapy should be maintained as secondary prophylaxis.

An asymptomatic patient with a solitary nodule or pulmonary cavitation due to *C. immitis* does not require specific antifungal therapy or resection. However, the development of complications due to the cavitation, such as local discomfort, bacterial or fungal superinfection or hemoptysis, necessitates initiation of azole therapy. Surgical resection of the cavities may be an alternative to chronic or intermittent antifungal therapy. Rupture of a coccidioidal cavity into the pleural space requires surgical intervention with closure by lobectomy with decortication, in addition to antifungal treatment.

For chronic fibrocavitary pneumonia, the initial treatment should be an oral azole with therapy for at least 1 year. If the disease persists, the alternatives are to switch to another oral azole, increase the dose if fluconazole was initially selected or administer amphotericin B. Surgical resection should be employed for patients with refractory focal lesions or severe hemoptysis.

The treatment of extrapulmonary, disseminated infection, without CNS involvement, is based on oral azole therapy, such as itraconazole or fluconazole (400 mg/day or higher in case of fluconazole). If there is little or no improvement or if there is vertebral involvement, treatment with amphotericin B is recommended (dosage similar to diffuse coccidioidal pneumonia). Concomitant surgical debridement or stabilization is also recommended. In patients with refractory coccidioidomycosis who have failed fluconazole, itraconazole, and amphotericin B products, treatment with posaconazole has been successful.<sup>89</sup>

For coccidioidal meningitis, lifetime treatment with azoles is indicated. Fluconazole, at doses of 800 mg per day or higher, has received the greatest attention in this regard.<sup>90</sup> There have been a few reports of successful treatment of coccidioidal meningitis with voriconazole.<sup>91,92</sup> Itraconazole has less utility in this role because of its irregular oral absorption. Obstructive hydrocephalus requires ventriculoperitoneal shunting. Intrathecal amphotericin B (0.01–1.5 mg) was previously a mainstay of therapy for meningeal coccidioidomycosis but toxicity limits its use and it is now usually reserved for patients with infection refractory to azoles.<sup>90</sup>

## Prevention

Among all the endemic mycoses, coccidioidomycosis has generated the most interest for the development of a vaccine, because it is known that prior infection engenders immunity and because the disease exists in a well-defined endemic zone. Many vaccine candidates have been proposed but no vaccine is commercially available.<sup>80</sup> For field workers in endemic areas, guidelines have been published to decrease their risk of acquiring coccidioidomycosis.<sup>66</sup>



## Paracoccidioidomycosis

### Etiology

Paracoccidioidomycosis (also called South American blastomycosis) is due to infection caused by *Paracoccidioides brasiliensis*. At 25–30°C the fungus grows slowly, producing a non-specific mycelial colony, with a white to buff-colored surface and a texture that ranges from glabrous to velvety, often with wrinkles and folds. The hyphae are septate and hyaline, with some containing intercalary chlamydoconidia or arthroconidia. Oval conidia (2–3 µm) arise from the sides of hyphae or short conidiophores. In tissue or enriched media at 37°C, conversion to the characteristic yeast form occurs over 10–20 days. The yeast colonies are white, with a butyrous, cerebriform aspect. The cells are spherical (3–30 µm, rarely up to 60 µm) with thin to moderately thick walls. At any point along the surface, spherical to lemon-shaped buds (2–10 µm diameter), with a narrow connection to the mother cell may develop, giving the characteristic “pilot wheel” appearance (Fig. 15-7).<sup>5,93,94</sup>

### Epidemiology and pathogenesis

The endemic area of paracoccidioidomycosis extends from Mexico to Argentina but the prevalence is higher in South America (see Table 15-1). The highest incidence is observed in Brazil, followed by Colombia, Venezuela, Ecuador and Argentina. Except for a single case in Trinidad, the disease has not been reported in the Caribbean, Nicaragua, Guayana or Chile. Patients diagnosed in areas outside Latin America had previous residence in endemic areas. The ecologic characteristics of the endemic areas are well defined: abundant forests and waterways, 90–180 cm of rainfall a year, short winters, and rainy summers. Paracoccidioidomycosis is rare in children and young adults but is regularly diagnosed in men older than 30 years. Although the rate of infection is equal in men and women, as shown by skin test with paracoccidioidin, progression towards symptomatic disease is more common in men.<sup>94</sup>

The organism has only rarely been isolated in nature. The environmental niche of *P. brasiliensis* remains poorly defined;

other than humans, naturally acquired infection has been demonstrated only in two armadillo species.<sup>95</sup> Most of those afflicted with paracoccidioidomycosis have been exposed in the course of agricultural activities. The disease is not transmissible person to person. The portal of entry for the fungus is either inhalation or traumatic inoculation. When inhaled, the organism converts into the yeast form in the lung parenchyma, which can subsequently disseminate to extrapulmonary sites.<sup>94</sup> Alcohol and tobacco use also play a role in the development of chronic paracoccidioidomycosis. The risk of becoming ill is 14 times greater among smokers and 3.6 times greater among persons with an alcohol intake of more than 50 g/day.<sup>96</sup> There has also been an increase in the number of reports involving immunocompromised patients, including those with AIDS.<sup>97,98</sup>

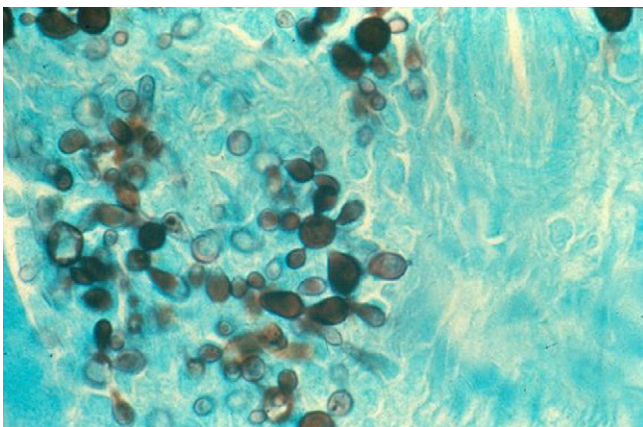
The host immune status is directly related to the clinical form and the severity of the mycosis. Cell-mediated immunity, involving macrophage activation by antigen-specific T lymphocytes, plays a critical role in providing resistance to this organism; both CD4 and CD8 cells play a part. Although specific antibodies are produced in large amounts, their role in host defense is uncertain.<sup>99</sup> Resistance to disease or a milder course is associated with a Th1 immune response (production of appropriate levels of interferon-γ and TNF-α), whereas a Th2 response (high levels of interleukin (IL)-4, IL-5, IL-10, TGF-β; impaired interferon-γ response) is associated with progressive disease.<sup>100</sup> Adult females are relatively protected from the disease, but not from the infection, by β-estradiol, which inhibits the transition of conidia-mycelial propagules to the yeast forms, a critical step in the pathogenesis.<sup>94,101</sup> Host genetics also influence susceptibility to paracoccidioidomycosis, including TNF-α and IL-10 gene polymorphisms and specific human leukocyte antigen (HLA) alleles.<sup>102,103</sup>

Both conidia and yeast cells of *P. brasiliensis* produce melanin, which contributes to virulence.<sup>104</sup> The major *P. brasiliensis* antigen is a glycoprotein termed gp43, which is also secreted in large amounts by the yeast form. This glycoprotein inhibits phagocytosis and the production of nitric oxide and H<sub>2</sub>O<sub>2</sub>.<sup>105</sup>

### Diagnosis

The most common diagnostic specimens from patients with suspected paracoccidioidomycosis are sputum, bronchoalveolar lavage fluid, pus from suppurating lymph nodes, CSF, and material from ulcerative lesions or tissue biopsies. The diagnosis is established when the characteristic pilot-wheel shape of the organism is observed by direct microscopic examination of specimens (KOH, Calcofluor, immunofluorescence) or in fixed samples (hematoxylin-eosin, methenamine silver, Papanicolaou, PAS). The presence of multiple budding distinguishes it from *C. neoformans* and *B. dermatitidis*. The mycelial form of *P. brasiliensis* is not diagnostic and conversion to the yeast form at 37°C is necessary for definitive identification. The identity of the mycelial form can also be confirmed using the exoantigen testing.<sup>5</sup> Currently, DNA probes for *P. brasiliensis* are not available. Suspected cultures should be handled in a biologic safety cabinet (class II agent).<sup>16</sup>

Detection of antibody by ID or CF using purified or recombinant antigens is useful in suggesting the diagnosis and may be used to evaluate response to therapy (see Table 15-2). It has been shown that a significant fall in antibody titer correlates with clinical improvement. During relapses, the levels of specific antibodies rise again and some patients who were



**Figure 15-7** *Paracoccidioides brasiliensis* yeast cells in tissue demonstrating “pilot wheel” appearance (GMS stain, 400×).

considered cured exhibit a residual reactivity. In the CF test, in contrast to the ID test, cross-reactivity with *H. capsulatum* antibodies occurs. Other serologic methods, including immunofluorescence, counterimmunoelectrophoresis, dot-blot, and ELISA, are also currently used. Several studies have shown that detection of antigens instead of antibodies is a powerful tool for early diagnosis and in cases of immunocompromised individuals, or when antibody detection is inconclusive. Several methods have been developed for the detection of antigens in sera and urine for patients with paracoccidioidomycosis.<sup>106-109</sup> The polymerase chain reaction has also been applied to its diagnosis.<sup>110</sup>

## Clinical findings

Most primary infections are self-limited, diagnosed only by a reactive skin test. The organism has the ability to remain dormant for long periods of time and can cause clinical disease at a later time if host defenses become impaired. In younger patients and immunosuppressed patients, the disorder is subacute and carries a dire prognosis. In these patients, pulmonary symptoms and radiographic changes are minimal and the most common clinical manifestations are bone marrow dysfunction, lymphadenopathy, and organomegaly. There may also be prominent intraabdominal manifestations, including diarrhea, jaundice, ascites, and intestinal obstruction and perforation.

In a recent series of children with paracoccidioidomycosis, satisfactory initial response to treatment was observed in only 56.7% of cases, half of the patients became asymptomatic only in the ninth month of treatment, and 17% of patients had at least one symptom remaining after 30 months of treatment.<sup>111</sup> In the adult form, most patients present with respiratory symptoms either as the sole manifestation of the disease or in addition to other signs and symptoms of the disease. The disease progresses slowly and may take months or even years to become established. Symptoms include a persistent cough with purulent sputum, chest pain, weight loss, weakness, malaise, dyspnea, and fever. Pulmonary lesions are nodular, infiltrative, fibrotic or cavitory. A paracoccidioidoma, a large cavitory mass, may also be seen. In approximately 25% of the patients, the lungs are the only organs affected (chronic unifocal). However, if not diagnosed and treated, infection can disseminate to extrapulmonary locations including the skin and mucosa, lymph nodes (especially cervical), adrenals, liver, spleen, central nervous system, and bones (chronic multifocal). The cutaneous lesions, usually verrucous, ulcerative or granulomatous, occur around the mouth and nose and may also affect the lower limbs. The ulcerated, painful mucosal lesions are usually located in the mouth or on the lips, gums, tongue, and palate.<sup>94</sup> The frequency of CNS involvement in different patient series ranges from 10% to 25% and manifests as seizures, hemiparesis, cerebellar signs, headache, hydrocephalus, paresthesias or confusion. Examination and culture of the CSF are frequently negative and the detection of gp43 antigen or antibodies to gp43 in the CSF is both sensitive and specific.<sup>112</sup>

Healing typically occurs with fibrosis of the affected tissues and in some cases, fibrosis may lead to cor pulmonale. The prominent fibrotic response observed in paracoccidioidomycosis may be due to the elaboration of TGF- $\beta$  by the host leukocytes; this cytokine plays an essential role in tissue repair but also promotes fibrosis.<sup>113</sup> The differential diagnosis

of paracoccidioidomycosis includes tuberculosis, histoplasmosis, neoplastic disorders including lymphoma, leishmaniasis, Hansen's disease, and syphilis.<sup>94</sup>

## Treatment

Itraconazole (100 mg/day for 6 months) is the drug of choice for the treatment of both adult and juvenile forms of paracoccidioidomycosis (see Table 15-3). Amphotericin B (total dose, 1.2–3.0 g) is reserved for refractory and/or severe cases and is followed by sulfonamide or azole therapy as the patient's condition stabilizes. Either sulfadiazine (4 g/day for adults and 60–100 mg/kg/day, in divided doses, for children) or one of the long-acting sulfa drugs (sulfamethoxypyridazine, sulfadimethoxine or trimethoprim-sulfamethoxazole) can be used. This dosage should be continued until clinical and mycologic improvement is apparent, at which time the dosage can be reduced by half. The long-acting compounds require 1–2 g/day for adults during the first 2–3 weeks of treatment; after clinical improvement (approximately 4 weeks), the dose can be decreased to 500 mg/day. Sulfonamide treatment should be continued for 3–5 years to avoid relapses, which occur in 20–25% of the cases.

Other azole compounds that can be used include ketoconazole (200–400 mg/day for 6–18 months) and fluconazole. Treatment with ketoconazole requires regular follow-up to monitor for hepatic and gonadal dysfunction. Fluconazole also has clinical activity against *P. brasiliensis*, although the need for higher doses (up to 600 mg/day), longer periods of therapy, and frequent relapses have limited its use. Along with antifungal treatment, appropriate supportive measures, such as improved nutrition and correction of anemia, are necessary to improve the clinical status of the patient.<sup>94,114,115</sup>

## Sporotrichosis

### Etiology

Sporotrichosis is caused by the fungus *Sporothrix schenckii*. Two reviews have been published recently.<sup>15,116</sup> At 25–30°C, the fungus grows as a mould. Colonies rapidly grow and are smooth and wrinkled with a dirty whitish color. The conidiogenous cells arise from undifferentiated hyphae, forming conidia in groups on small, clustered conidia. Conidia are unicellular, tear-shaped to clavate, and occur singly or in chains. Often thin- or thick-walled, hyaline or brown conidia arise alongside the hyphae. In vivo and in supplemented agar, such as BHI or BCG agar at 37°C, *S. schenckii* exists as a yeast. In this form, the colonies are off-white to beige with a creamy texture and the organism reproduces by budding. The yeast form is 4–6  $\mu$ m in diameter and is often cigar-shaped. Some strains grow best at temperatures below 35°C and are usually found in fixed cutaneous lesions.<sup>117,118</sup>

### Epidemiology and pathogenesis

Infection with *S. schenckii* typically occurs by direct skin inoculation from contaminated soil or thorny plants. Zoonotic transmission from scratches or bites from cats and armadillos has also been reported.<sup>116</sup> Rarely, infection occurs via the

respiratory tract. Outbreaks of infection have been associated with contaminated plant material such as straw, wood, hay bales and sphagnum moss. The largest outbreak in the United States occurred in 1988 and involved 84 patients in 15 states who handled conifer seedlings.<sup>116</sup> Most cases are associated with occupational or recreational exposures and the disease occurs worldwide, but a preponderance of cases has been reported from North and South America (see Table 15-1).<sup>116</sup> A recent epidemiologic study of 304 sporotrichosis cases from Brazil showed a male predominance (68%), with a median age of 44 years; the majority of cases occurred in farmers (55%) or other persons with outdoor occupations (19%). However, 26% of cases occurred in persons with no obvious soil/plant exposure.<sup>119</sup>

The cellular response to *S. schenckii* infection is both neutrophilic and monocytic. Cell-mediated immunity is important to resolution of the infection. The humoral immune response does not provide protection against infection.<sup>15,120</sup> The cell wall lipid components of the fungus may contribute to virulence by inhibiting macrophage phagocytosis.<sup>121</sup> Melanin is another virulence factor.<sup>116</sup> Recently, the disease has been reported in a patient receiving TNF- $\alpha$  antagonists and in a patient taking inhaled corticosteroids, again demonstrating the importance of cell-mediated immunity in defense against the fungus.<sup>122,123</sup> Laboratory-acquired infection has occurred and so cultures must be handled with care (class II agent).<sup>124</sup>

## Diagnosis

The diagnosis is established by culture of the organism from the site of infection. Sputum, synovial fluid, CSF, biopsy specimens, exudates and, rarely, blood have been reported to yield *S. schenckii* when cultured. Growth of the organism may occur within 3–5 days. Histopathologic analysis demonstrates the typical cigar-shaped yeasts that may be surrounded by a stellate, PAS-positive material known as an asteroid body. Culture is superior to histopathologic methods in terms of diagnostic yield, because the organism is not abundant in tissues and is visualized poorly with hematoxylin-eosin and PAS stains.<sup>116</sup> A latex agglutination test for detection of antibodies against *S. schenckii* is available, although not used widely. Antigen-based testing is not available (see Table 15-2).<sup>15,118</sup>

## Clinical findings

Sporotrichosis can be classified into four categories: (1) lymphocutaneous; (2) fixed cutaneous; (3) disseminated; and (4) extracutaneous.<sup>116</sup> The lymphocutaneous form is the most common, comprising over 75% of cases. Typically after an incubation period of 1–10 weeks or longer, reddish-purple, necrotic, nodular cutaneous lesions appear that follow the lymphatics and commonly ulcerate (Fig. 15-8). Involvement of distal extremities may be related to the intolerance of some strains to temperatures of 37°C.<sup>11</sup> Direct spread of the organism to joint or bone is occasionally seen.<sup>125</sup> In the fixed cutaneous form, the lesion is confined to the site of the initial inoculation. However, the lesion does not resolve spontaneously. Erythema nodosum may accompany the cutaneous forms of sporotrichosis. Disseminated infection such as visceral, osteoarticular, meningeal and pulmonary sporotrichosis



**Figure 15-8** Lymphangitic spread of cutaneous sporotrichosis.

is often seen in patients with underlying diseases, including alcoholism, diabetes mellitus, hematologic malignancy, the use of immunosuppressive agents, chronic obstructive pulmonary disease, and HIV infection.

The extracutaneous form is rare and results from inhalation of conidia or hematogenous seeding from a deep inoculation.<sup>116</sup> Osteoarticular disease with monoarthritis or tenosynovitis is common for extracutaneous sporotrichosis.<sup>116,125</sup> Pulmonary sporotrichosis typically affects men with underlying lung disease and resembles tuberculosis, with fibrocavitary complications.<sup>116</sup> Sporotrichotic meningitis is rare but has been described in HIV patients with a CD4 count <200 cells/ml.<sup>116</sup>

## Treatment

Practice guidelines for the treatment of sporotrichosis were published in 2007.<sup>126</sup> All forms of cutaneous sporotrichosis require treatment with antifungal or other local measures (see Table 15-3). In patients with cutaneous or lymphocutaneous sporotrichosis, itraconazole 200 mg/day is recommended for 2–4 weeks after all lesions have resolved, usually for a total of 3–6 months. Patients who do not respond should be given a higher dose of itraconazole (200 mg twice daily); terbinafine, at a dosage of 500 mg twice daily; or a saturated solution of potassium iodide (SSKI), initiated at a dosage of five drops (using a standard eye-dropper) three times daily and increasing, as tolerated, to 40–50 drops three times daily. Fluconazole (400–800 mg daily) should be used only if the patient cannot tolerate these other agents. Local hyperthermia can be used to treat fixed cutaneous lesions in patients (such as pregnant or nursing women) who cannot safely receive any of the other agents.

For patients with extensive or life-threatening pulmonary sporotrichosis, the drug of choice is amphotericin B, given as a lipid formulation, at a dosage of 3–5 mg/kg/day, or amphotericin B deoxycholate at a dosage of 0.7–1.0 mg/kg daily, as initial therapy. After a favorable response, itraconazole 200 mg twice daily can be used to complete a total of at least 12 months of therapy. For less severe disease, itraconazole 200 mg twice daily can be used for initial therapy and continued for at least 12 months.

The preferred treatment for osteoarticular involvement is itraconazole 200 mg twice a day for at least 12 months.



Amphotericin B, given as a lipid formulation at a dosage of 3–5 mg/kg/day or amphotericin B deoxycholate at 0.7–1.0 mg/kg/day, is indicated in those patients with extensive involvement or for those patients for whom itraconazole therapy fails.

For meningeal sporotrichosis, the treatment of choice is amphotericin B as a lipid formulation at a dosage of 5 mg/kg/day for 4–6 weeks, recommended for initial therapy followed by itraconazole 200 mg twice daily as step-down therapy. Amphotericin B deoxycholate as initial therapy was not favored by the guidelines committee in this setting.<sup>126</sup> For patients with AIDS, suppressive therapy with itraconazole at 200 mg/day is recommended to prevent relapse but there are no guidelines for the discontinuation of antifungal therapy for the patient who has achieved immune reconstitution with antiretroviral therapy.

For pregnant women with disseminated or pulmonary infection, amphotericin B should be used, preferably with a lipid formulation, at a dosage of 3–5 mg/kg/day or alternatively amphotericin B deoxycholate at 0.7–1.0 mg/kg/day; azoles should be avoided. In localized, cutaneous disease, there is no risk of the infection disseminating to the fetus, nor is sporotrichosis worsened during pregnancy; thus, there is little risk involved in delaying treatment, although local hyperthermia can be initiated. Children can be treated with itraconazole 6 mg/kg up to 400 mg/day or SSKI, initiated at one drop three times daily, up to a maximum of one drop per kg of body weight or 40–50 drops three times daily, whichever is lowest; amphotericin B deoxycholate (0.7–1.0 mg/kg/day) can be used in disseminated infection.<sup>15,116,126</sup>

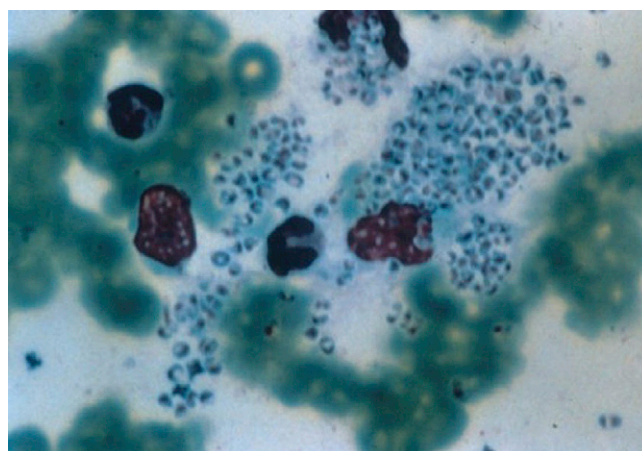
There are no published data on the use of the newer azoles, voriconazole or posaconazole, for sporotrichosis. Voriconazole is not active in vitro against *S. schenckii* whereas posaconazole does have activity in vitro.<sup>126</sup>

## Penicilliosis<sup>127</sup>

### Epidemiology and pathogenesis

Penicilliosis marneffei, due to *Penicillium marneffei*, is an infection endemic to southeast Asia (Thailand, Vietnam, Myanmar, Hong Kong, Indonesia, Laos, Malaysia, Singapore, Taiwan, the Manipur state of India, and the Guangxi province of China) (see Table 15-1). Imported cases in Europe and the US have also been reported. Infection is more common in the immunosuppressed population, mostly HIV patients, and penicilliosis is the third most common presenting opportunistic infection in Northern Thailand. *P. marneffei* has been isolated from the organs of bamboo rats (*Cannomys badius*) but is rarely isolated from soil.<sup>127–129</sup> Human-to-human transmission does not occur. The conidia of the mycelial form can cause laboratory-acquired infections in immunosuppressed health-care personnel (class II agent).<sup>130</sup>

The pathogenesis of penicilliosis has been poorly studied. Though the disease can be localized, it is more likely to be disseminated in both normal and immunocompromised hosts. Infection is thought to occur through inhalation of the conidia or through cutaneous inoculation. The organisms are engulfed by macrophages, in which they multiply intracellularly and transform into yeasts.<sup>127</sup> Melanin is produced by



**Figure 15-9** *Penicillium marneffei* yeasts on Wright stain of bone marrow demonstrating budding with clearing of cross-walls from fission of cells (400×).

both conidia and yeast and may act as a virulence factor.<sup>131</sup> Dissemination of infection occurs through the lymphatics or hematogenously. The reticuloendothelial system is predominantly involved. The infection can remain dormant and reactivate later. In the immunocompetent host, the cell-mediated immune response is prominent with the formation of epithelioid granulomas that are indistinguishable from tuberculous granulomas.<sup>132</sup> In a murine model of infection, wild-type mice controlled the infection with granuloma formation and the elaboration of a Th1 immune response.<sup>133</sup> There is no evidence for a role of humoral immunity in the resolution of penicilliosis marneffei infection. In patients with HIV infection, the histopathologic features depend on the degree of immunosuppression.<sup>134</sup> At lower CD4 cell counts, the disease is more invasive. The blunted immune response is reflected by an absence of granulomas and extensive proliferation of extracellular yeast and intracellular forms within foamy macrophages.<sup>135</sup> Yeast forms can occasionally be demonstrated in peripheral blood.<sup>127</sup>

### Diagnosis

The diagnosis is established by smears of bone marrow, exudates from ulcerative skin lesions, lymph node aspirates or buffy coat of blood, which show elliptical yeasts, 2–3 × 6–8 μm, inside phagocytes, resembling *H. capsulatum* except that prominent cross-walls may be seen (Fig. 15-9). It can also be occasionally confused with *Cryptococcus neoformans* or *Leishmania* spp. Definitive diagnosis relies upon isolation of *P. marneffei* from clinical specimens. Culture at 30°C produces a mould with sporulating structures typical of *Penicillium*. Identification is aided by the formation of a soluble red pigment that diffuses into the agar. This dimorphism is not found in other known members of the genus *Penicillium*.<sup>136,137</sup>

Specific fluorescent antibody examination of tissue samples has been described. A few serodiagnostic techniques have been described; however, these tests need further evaluation before use in clinical practice.<sup>127</sup> Antigen detection assays with high sensitivity and specificity have been developed but are not

commercially available.<sup>17</sup> PCR-based assays of clinical samples for the rapid diagnosis of *penicilliosis marneffei* have also been described (see Table 15-2).<sup>127</sup>

## Clinical findings

The organism is acquired through inhalation of conidia and disseminated infection develops. Signs and symptoms include fever, anemia, leukopenia, thrombocytopenia, weight loss, diarrhea, hepatosplenomegaly, generalized lymphadenopathy, cough, pulmonary infiltrates, and a characteristic rash resembling molluscum contagiosum (umbilicated papules), predominantly on the face and trunk. The presentation may mimic tuberculosis, melioidosis, leishmaniasis, and other AIDS-related opportunistic infections, such as histoplasmosis and cryptococcosis.<sup>127,138</sup>

## Treatment

Therapy with amphotericin B (0.6 mg/kg/day) for 2 weeks, with or without flucytosine, followed by itraconazole 200 mg twice daily for 10 weeks, is effective for the treatment of disseminated infection (see Table 15-3). Fluconazole therapy has been associated with a high rate of failure. Itraconazole is useful to prevent relapses of this disease in patients with advanced AIDS.<sup>138</sup> However, antifungal therapy can be successfully discontinued in HIV patients on antiretroviral therapy who achieve CD4 counts above 100 cells/ $\mu$ l.<sup>139</sup> A recent study of the susceptibility of the fungus to amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole, and posaconazole showed that the latter two agents had the lowest MIC values, 0.03 and 0.001  $\mu$ g/ml, respectively. However, there is no clinical experience with these agents.<sup>140</sup>

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# Dermatophytes and dermatophytoses

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## Definition

Dermatophytes are a unique group of fungi that infect keratinous tissue and are able to invade the hair, skin, and nails of a living host. This closely related group of organisms can be categorized into one of three genera: *Trichophyton*, *Microsporum*, and *Epidermophyton*. Species within these genera that do not invade keratinous tissue are by definition excluded from the dermatophytes. As with a number of fungi, dermatophytes may exhibit two phases in their life cycle: the anamorph state (imperfect or asexual phase) that is the state isolated in the laboratory, and the teleomorph state (perfect or sexual phase). The teleomorphs for all dermatophyte species have not yet been identified, but the generic name for both *Trichophyton* and *Microsporum* is *Arthroderma* (the obsolete name *Nannizzia* has been found to be identical).<sup>1-3</sup>

## Natural habitat

Dermatophytes are broadly classified into three groups on the basis of their natural habitats. With the exception of *M. gypseum*, geophilic species (soil-inhabiting saprophytes) are only occasionally pathogenic for man or animals. Zoophilic species are those whose normal host is animals but which may also infect man. These are thought to have developed the ability to hydrolyze keratinous debris in the soil and evolved into “keratinophilic fungi” that parasitized animal hosts. Examples include those species with an affinity for a single animal host such as *T. equinum* (horse) and *T. verrucosum* (cattle), and those that may infect a wide range of animals (*T. mentagrophytes* and *M. canis*). Anthropophilic species, most notably *T. rubrum*, are believed to have evolved from zoophilic fungi and are confined to man as a host.<sup>2-5</sup>

## Geographic distribution

Although dermatophytes generally have worldwide distribution, some species are restricted to a specific geographic location. For example, *T. concentricum*, the causative agent of imbricata, is endemic to the South Pacific and certain regions of South America and has seldom been isolated in Europe.<sup>6</sup>

A summary of clinical isolates recovered from one reference laboratory showed that *T. rubrum* remains the most prevalent dermatophyte pathogen, and increased incidence of this species was observed in onychomycosis, tinea corporis, tinea cruris, tinea manuum, and tinea pedis.<sup>7</sup>

Reports have shown *Trichophyton tonsurans* to be the main causative organism infecting children with tinea capitis in the United States, achieving near exclusionary proportions and confirming data from a two-decade survey (1961–1980) conducted in Chicago.<sup>7-9</sup> Similarly, *T. tonsurans* is the dominant dermatophyte infecting the hair and scalp of children in Canada, replacing *T. verrucosum*, *M. canis*, and *M. audouinii*.<sup>10</sup> Conversely, *M. canis* is more common in Europe, though the largest increase in recent years has been in *T. tonsurans*.<sup>10-16</sup> *T. tonsurans* and *M. canis* are both commonly found in Puerto Rico and South America.<sup>17-21</sup> In the Middle East, the predominant organisms are *M. canis*, *T. tonsurans*, and *T. violaceum*, while *T. violaceum* is reported from >90% of tinea capitis cases across Africa.<sup>22-28</sup> Similarly, the main infecting agent in children with tinea capitis in India and Nepal is also *T. violaceum*.<sup>29-32</sup> Understanding the epidemiology of these dermatophytes is essential, since management of infections caused by different genera may differ, and cures for different dermatophytes species vary, with *Microsporum* often involved in recalcitrant infections.<sup>33,34</sup>

## Clinical manifestations

Dermatophyte infections have different clinical manifestations in different areas of the body. Naming of the clinical disease is done by appending a Latin term for the specific body part to the word tinea.<sup>35-38</sup>

### Tinea pedis

Tinea pedis, or infection of the feet, can involve the interdigital web spaces and soles, or the sides of the feet (“moccasin” type). It may have an acute onset, with inflammation, vesicles, and pustules. The most common etiologic agents are *E. floccosum*, *T. mentagrophytes*, and *T. rubrum*. It is thought that untreated tinea pedis may lead to nail involvement, or onychomycosis.



## Tinea unguium

Tinea unguium is defined as fungal infection of the nail caused by dermatophytes (whereas onychomycosis refers to any fungal infection of the nail). Distal subungual onychomycosis begins under the leading edge of the nail or along the lateral edges and is characterized by the accumulation of crumbling subungual debris and thickened, discolored nails. In proximal subungual onychomycosis, infection begins at the proximal nailfold with infection of the nail matrix. The most common cause of both types is *T. rubrum*, although *T. mentagrophytes* and *E. floccosum* are also frequently isolated from nail specimens. Superficial white onychomycosis, characterized by white patches on the surface of the nail, is caused primarily by *T. mentagrophytes*.

## Tinea capitis

In tinea capitis, infection of the hair shaft distinguishes scalp infection from that on glabrous skin. Dermatophyte species may be either ectothrix, in which spores cover the surface of the hair shaft, or endothrix, in which hyphae form arthrospores within the hair shaft. *M. canis* and *M. audouinii* are examples of ectothrix infection, with the latter causing a pronounced inflammatory response. *T. tonsurans* causes endothrix hair invasion often referred to as “black-dot” because hairs break off close to the surface. Kerion is an inflammatory pustular folliculitis involving circumscribed scalp areas, most commonly caused by *T. verrucosum*. Kerion differs from favus, in which pus from hair follicles forms a crust, or scutula, along the hair shaft. Long-standing favus, caused by *T. schoenleinii*, can lead to scarring and hair loss.

Tinea barbae, involving the hairs of bearded areas and showing symptoms similar to tinea capitis, is most often caused by *T. mentagrophytes* and *T. verrucosum*.

## Tinea corporis

Tinea corporis refers to annular lesions with raised borders originating on the glabrous skin. Lesions may be singular, multiple or confluent, and exhibit a range of inflammatory responses, ranging from scaling and minimal erythema to highly inflammatory lesions composed of pustules, vesicles, and marked erythema. Inflammation is often greatest at the advancing edge of the lesion, with a certain amount of central clearing. The most common causes are *T. rubrum*, *T. mentagrophytes*, and *M. canis*. Infection of hair follicles within the lesion can lead to a deep dermal inflammatory reaction similar to kerion of the scalp, termed Majocchi's granuloma. Tinea imbricata is caused by *T. concentricum*, which causes concentric rings of scaling which spread peripherally over many years.

## Specimen collection

### Tinea unguium

In distal subungual nail infections, debris from the junction of the nail and nail bed is scraped and collected for direct examination and culture. Often, the culture will be negative for the presence of dermatophytes even when the specimen is positive

on direct examination, due to the presence of non-viable septate hyphae in the distal portion of the nail.<sup>39</sup>

In white superficial onychomycosis, the surface dorsal layers are scraped with a scalpel and shavings submitted for mycologic testing.<sup>40-42</sup>

### Obtaining nail specimens

1. Clean affected toenails and fingernails with a premoistened alcohol prep. If the nail is heavily soiled, wash with soap and water first.
2. Clip the nail as far back as possible without excessive patient discomfort – discard nail clippings.
3. Obtain the crumbling subungual debris from under the trimmed edge of the nail by scraping with a 1 mm or 2 mm serrated curette, or a 15 blade scalpel.
4. Collect the sample in a cardboard folder such as a Dermapak (Dermaco, UK), fold according to label directions, and record patient name, physician name, and date of collection on the reverse.
5. Do not use plastic bags or containers to collect subungual debris, as static electricity will cause the specimen to adhere to the plastic.

## Tinea capitis, tinea barbae

Hairs and skin scrapings should be sampled from non-inflammatory areas of the scalp, as the inflammatory response may cause non-viability of fungal spores.<sup>43</sup> The use of a Wood's lamp that emits ultraviolet light at a wavelength of 365 nm will cause lesions of certain pathogenic dermatophytes, including *M. canis* and *M. audouinii*, to fluoresce in a darkened room.<sup>44</sup> Note, however, that *T. tonsurans*, the predominant organism involved in tinea capitis in the United States, does not fluoresce.

### Obtaining hair specimens

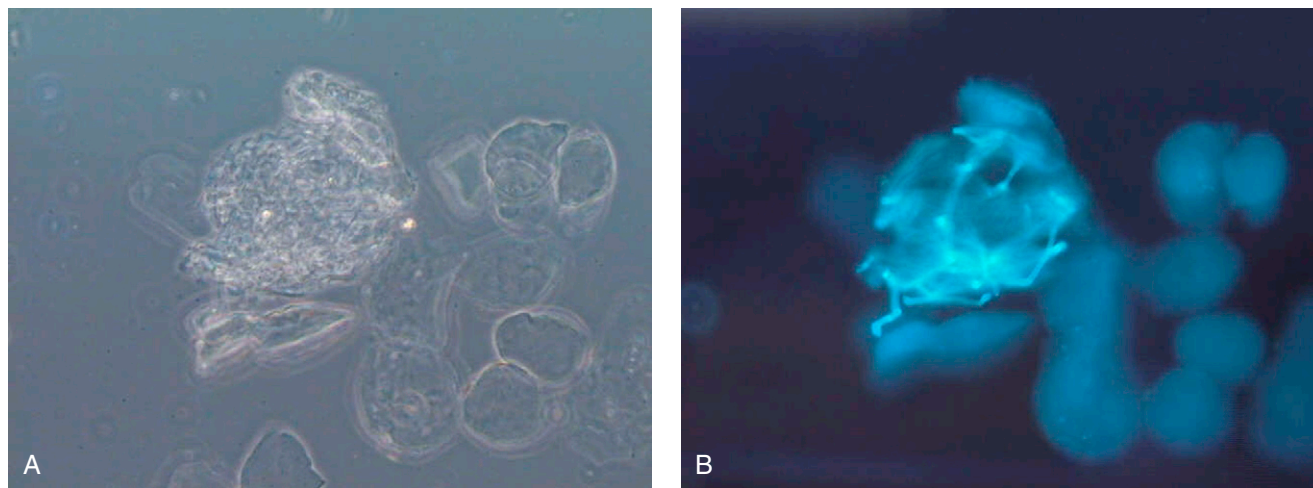
1. Trim hairs close to the root and pluck hairs with tweezers. (Do not submit long strands of hair.)
2. Scalp skin specimens may be collected by scraping with a toothbrush or the edge of a clean glass slide or scalpel.
3. Loose hairs and scalp scrapings may be submitted in a Dermapak.
4. Pustules may be swabbed with a cotton-tipped applicator and sent dry to the laboratory. If using a culturette, do not crush the ampule at the base of the container – the cotton tip should remain dry.

## Tinea corporis, tinea cruris

Samples of scale and vesicle fluid should be taken from the outer margin of skin lesions, as the number of viable fungal elements is decreased in the center as healing occurs.<sup>44</sup>

### Obtaining skin specimens

1. Clean affected areas gently with soap and water or an alcohol prep to reduce bacterial contamination. Allow the area to dry thoroughly.
2. With a #15 blade or edge of a glass microscope slide, gently collect 10–15 pieces of scale from the raised edges of the lesions directly onto a Dermapak.
3. If blisters are present, the roof of the blister can be collected and sent in the Dermapak.



**Figure 16-1** Septate hyphae in subungual debris, viewed under (A) white and (B) ultraviolet light with Calcofluor white stain (40 $\times$ ). (Images courtesy of Center for Medical Mycology.)

**Note:** Specimens may be shipped at room temperature to the laboratory. International Air Transport Association (IATA) regulations, including triple-layer packaging, must be followed when shipping by mail.

## Laboratory examination

### Direct examination

The potassium hydroxide (KOH) preparation is used to clear clinical material in order that fungal elements can be seen more easily. In this method, a small portion of clinical sample is added to 10% KOH on a glass slide with a glass coverslip and left to sit at room temperature for 3 minutes to allow for digestion of host cells. Slides are then examined microscopically at 400 $\times$  under phase-contrast microscopy for the presence of hyaline septate hyphae (2–4  $\mu$ m in diameter) or fungal conidia, indicating the presence of fungal disease.<sup>45,46</sup> Nail samples infected with non-dermatophyte moulds such as *Scytalidium*, *Scopulariopsis brevicaulis*, and certain *Aspergillus* spp. will often demonstrate irregular filaments, swollen nodules, etc. that can often be distinguished from the cylindric filaments or regular chains of spherical conidia exhibited by dermatophyte species.<sup>46–48</sup> The observer must also be careful to distinguish fungal elements from artifacts such as crystals, cloth fibers, and elastin fibers from the cutis. The use of Calcofluor white stain, a fluorescent dye that targets chitin in the fungal cell wall, increases the sensitivity of the direct exam; however, this requires a microscope equipped with a mercury vapor lamp and broadband excitation filters to achieve radiation in the range of 300–412 nm<sup>49</sup> (Fig. 16-1).

### Culture methods

The remainder of the clinical sample should be plated onto selective and non-selective fungal media. Media selective for dermatophytes, such as Mycosel and Mycobiotic agar (Becton Dickinson, Franklin Lakes, NJ), contain cycloheximide for the inhibition of saprophytic moulds, e.g., *Penicillium* and

*Aspergillus*. Non-selective media, such as potato dextrose agar and Sabouraud dextrose agar, may have antibiotics added to inhibit bacterial contamination. Dermatophyte test medium (DTM), which is supplemented with cycloheximide, gentamicin, and chlortetracycline, was designed as a rapid test for dermatophytes; however, the use of this medium is not recommended, as many non-dermatophytes will also turn this medium red (giving false-positive results), and isolates often exhibit atypical colonial and microscopic characteristics when grown on DTM.<sup>50</sup> Cultures should be grown in screw-capped tubes or Petri plates sealed with parafilm or other gas-permeable tape. Cultures from clinical samples for dermatophyte isolation should be incubated at 30°C for a total of 4 weeks before being considered mycologically negative. However, the majority of cultures will become positive within 1–2 weeks.

Dermatophyte isolates can be identified to genus/species by colonial appearance, microscopic examinations by Scotch tape preparation, and biochemical tests such as growth patterns on *Trichophyton* agars and urease.<sup>51,52</sup>

### Scotch tape preparation

Fungal isolates are often identified by distinguishing microscopic characteristics such as the appearance of the hyphae and the production of conidia. The Scotch tape preparation is an easy means of examining a fungal colony for microscopic structures.<sup>51</sup>

#### Procedure

1. Cut a piece of clear Scotch tape and fold it back on itself with the adhesive side turned outward, holding it between the thumb and middle finger.
2. Using the index finger, press the adhesive side of the tape onto the surface of the colony and pull it away. The aerial hyphae of the colony will stick to the adhesive surface.
3. Place the tape adhesive side down into a drop of lactophenol-cotton blue or KOH previously placed on the center of a glass slide.
4. Examine microscopically for septate hyaline hyphae, chlamydoconidia, microconidia, and/or macroconidia.

*Trichophyton* agar slants

The *Trichophyton* agar slants are a series of media containing different vitamins or amino acids used to differentiate between *Trichophyton* species (Table 16-1). The requirements of different dermatophyte isolates for these compounds are demonstrated by the enhancement of growth on the supplemented media, as compared to poorer growth on the corresponding basal control medium.<sup>51</sup> For example, *T. tonsurans* will exhibit more luxuriant growth on *Trichophyton* #4 agar than on *Trichophyton* #1 because of a partial requirement for thiamine (Table 16-2).

## Procedure

1. Using a dissecting needle, inoculate the surface of the *Trichophyton* agar slant with a small portion of an actively growing colony. Care must be taken not to transfer agar from the culture plate because nutrients in the agar may give false-positive results.

2. Incubate the slants at 30°C for 7–14 days. (If the isolate is suspected to be *Trichophyton verrucosum*, demonstrating long chains of chlamydoconidia and antler-like branches under microscopic examination, incubate the slants at 37°C.)
3. Grade the amount of growth on the surface of the slants as follows:
  - 4+ good growth
  - 2+ intermediate growth
  - + trace
  - 0 absence of growth

## Hydrolysis of urea

Christensen's urea agar slants are used to differentiate between *Trichophyton* species. Urea hydrolysis in Christensen's medium causes a rise in pH, which causes a color change indicating the formation of ammonia.<sup>51</sup> For example, *T. mentagrophytes* will produce a bright pink color (positive), while *T. rubrum* will produce no color change (negative) (see Table 16-2).

## Procedure

1. Using a dissecting needle, inoculate the surface of the urea agar slant with a small portion of an actively growing colony.
2. Incubate the slants at 30°C for 7 days.
3. A positive reaction is indicated by a change of the original yellow color to bright pink.

## Hair Perforation Test

Some species of dermatophytes, such as *Trichophyton mentagrophytes*, *Microsporum canis*, and *Microsporum gypseum*, produce specialized hyphae called “perforating organs” capable of perforating hair in vitro. The hair perforation test is a diagnostic test to differentiate these species from other dermatophyte species that do not perforate hair in vitro.<sup>53</sup>

## Procedure

1. Make a lawn of the test isolate on the surface of a potato dextrose agar plate.
2. Place several sterile hairs, taken from a prepubescent blonde child, onto the fungal lawn.

**Table 16-1** Composition of *Trichophyton* Agars

<i>Trichophyton</i> Agar #	Composition
1	Vitamin-free casamino acids agar (base)
2	Agar #1 + inositol
3	Agar #1 + inositol + thiamine
4	Agar #1 + thiamine
5	Agar #1 + nicotinic acid (niacin)
6	Vitamin-free ammonium nitrate agar (base)
7	Agar # 6 + histidine

**Table 16-2** Growth patterns of common *Trichophyton* spp. on *Trichophyton* Agars

Species	Agar #							Urea
	1	2	3	4	5	6	7	
<i>T. rubrum</i>	4+	4+	4+	4+				-
<i>T. tonsurans</i>	1+	1+	4+	4+				+
<i>T. mentagrophytes</i>	4+	4+	4+	4+	4+			+
<i>T. megninii</i>						1+	4+	+
<i>T. soudanense</i>	1+	1+	1+	1+				-
<i>T. verrucosum</i> (84% of isolates)	0	±	4+	0				-
<i>T. violaceum</i>	±	±	4+	4+				-



3. Incubate at 30°C for up to 28 days. Examine every 7 days.
4. Mount some of the hairs in a drop of lactophenol-cotton blue between a slide and a coverslip.
5. The test is positive when cone-shaped perforations are observed perpendicular to the long axis of the hair (Fig. 16-2).

## Identification of dermatophyte species

This chapter is not intended as an exhaustive review of the dermatophyte species; only a sampling of the most commonly isolated species will be described. The reader is referred to several excellent textbooks describing the dermatophytes in greater detail.<sup>46,51,52</sup>

### *Trichophyton rubrum* (Fig. 16-3)

#### Colonial morphology

The surface is granular or fluffy, white to buff. The reverse is deep red or purplish or occasionally brown, yellow-orange or colorless. Pigment production is better seen on potato dextrose agar than Mycosel agar.

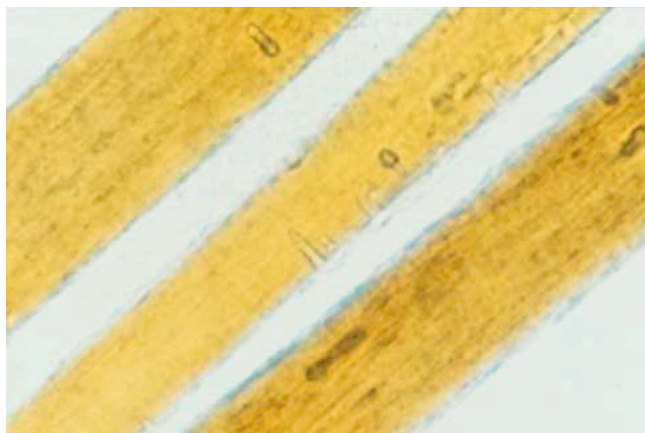
#### Microscopic morphology

Hyphae are septate. Tear-shaped microconidia (2–3.5 × 3–5.5 µm) usually form singly along the sides of the hyphae. Macroconidia (4–8 × 40–60 µm) are narrow and thin-walled, with parallel sides and 4–10 cells. They may be abundant, rare or absent.

### *Trichophyton mentagrophytes* (Fig. 16-4)

#### Colonial morphology

The surface may be tan and powdery, becoming yellowish, or white and downy. The powdery form exhibits concentric and radial folds. Reverse is usually brownish tan but may be colorless, yellow or red.



**Figure 16-2** *T. mentagrophytes* demonstrating positive hair perforation (40×). (Image courtesy of Center for Medical Mycology, Cleveland, Ohio, USA.)

#### Microscopic morphology

Hyphae are septate. Microconidia in powdery cultures are very round (4–6 µm in diameter) and clustered on branched conidiophores or, in fluffy strains, are smaller, fewer in number, and tear shaped. Macroconidia are sometimes present; they are cigar-shaped and thin-walled (4–8 × 20–50 µm), have narrow attachments to hyphae, and contain 1–6 cells. Coiled spiral hyphae are often seen.

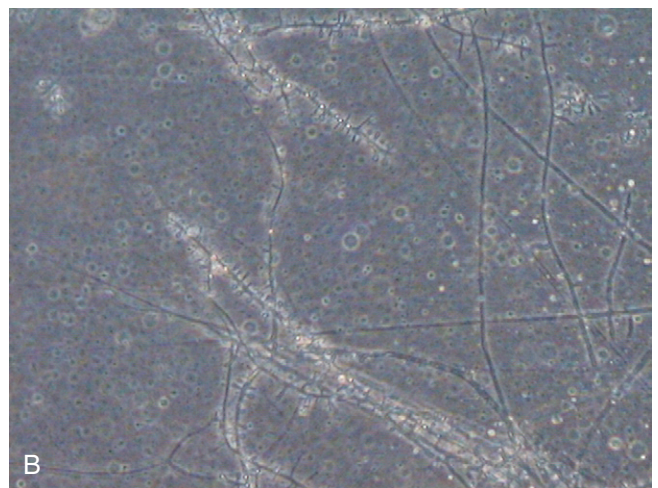
### *Trichophyton tonsurans* (Fig. 16-5)

#### Colonial morphology

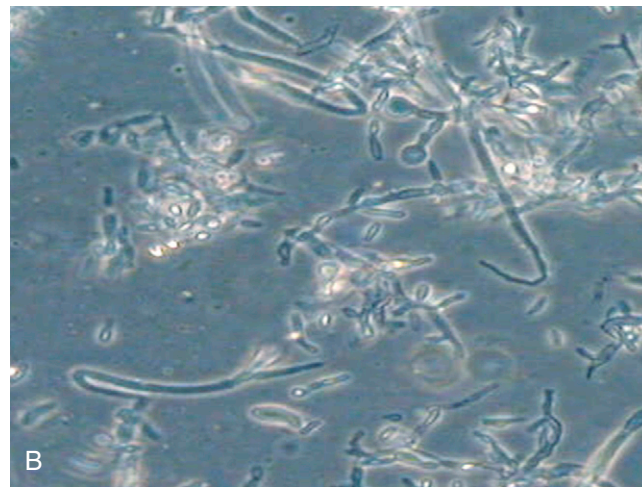
Colonies may be white, yellow, rose or brown. The surface is usually suede-like, with radial or concentric folds. The reverse is usually reddish brown, with pigment occasionally diffusing into the medium.

#### Microscopic morphology

Hyphae are septate, with many conidia formed along the hyphae or on short perpendicular conidiophores. Microconidia are teardrop or club shaped, but may enlarge to form “balloon” forms. Intercalary and terminal chlamydoconidia are common. Macroconidia are rare, irregularly shaped and slightly thick-walled.



**Figure 16-3** *Trichophyton rubrum*. (A) Colony surface. (B) Microscopic (40×). (Image courtesy of Center for Medical Mycology, Cleveland, Ohio, USA.)



**Figure 16-4** *Trichophyton mentagrophytes*. (A) Colony surface. (B) Microscopic (40 $\times$ ). (Image courtesy of Center for Medical Mycology, Cleveland, Ohio, USA.)

### ***Epidermophyton floccosum* (Fig. 16-6)**

#### Colonial morphology

The surface of the colony is brownish yellow to olive gray. It is folded in the center with radial grooves, becoming velvety. The reverse of the colony is orange to brown, sometimes with a thin yellow border.

#### Microscopic morphology

Hyphae are septate; no microconidia are produced. Macroconidia are smooth, thin- to slightly thick-walled, and club shaped with rounded ends. They contain 2–6 cells, and are found singly or in characteristic clusters.

### ***Microsporum canis* (Fig. 16.7)**

#### Colonial morphology

Surface is white, coarsely fluffy or furry, with yellow pigment at the edges and closely spaced radial grooves. The reverse is deep yellow to brown.

**Figure 16-5** *Trichophyton tonsurans*. (A) Colony surface. (B) Microscopic (40 $\times$ ). (Image courtesy of Center for Medical Mycology, Cleveland, Ohio, USA.)

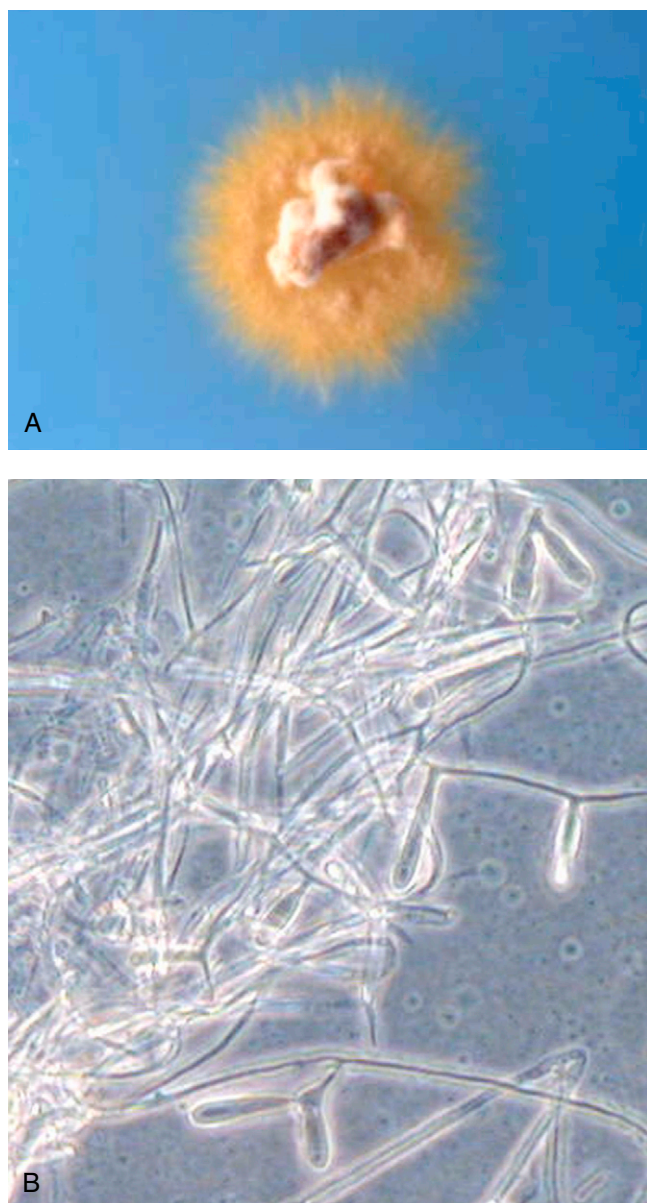
#### Microscopic morphology

Hyphae are septate with numerous spindle-shaped, rough thick-walled macroconidia (10–25  $\times$  35–110  $\mu$ m). The macroconidia characteristically taper to spiny, bent knob-like ends that resemble “dog snouts.” Microconidia, club shaped and smooth walled, form sparsely along the hyphae.

## **Susceptibility testing of dermatophytes**

The Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi (M38-A)<sup>54</sup> does not address the antifungal susceptibility of the dermatophyte *Trichophyton*, *Microsporum*, and *Epidermophyton* species, in which conidia formation is sometimes problematic. As part of the CLSI subcommittee’s efforts to standardize antifungal susceptibility testing, two interlaboratory studies were conducted to determine the reproducibility of the minimum inhibitory concentration (MIC) testing method of common

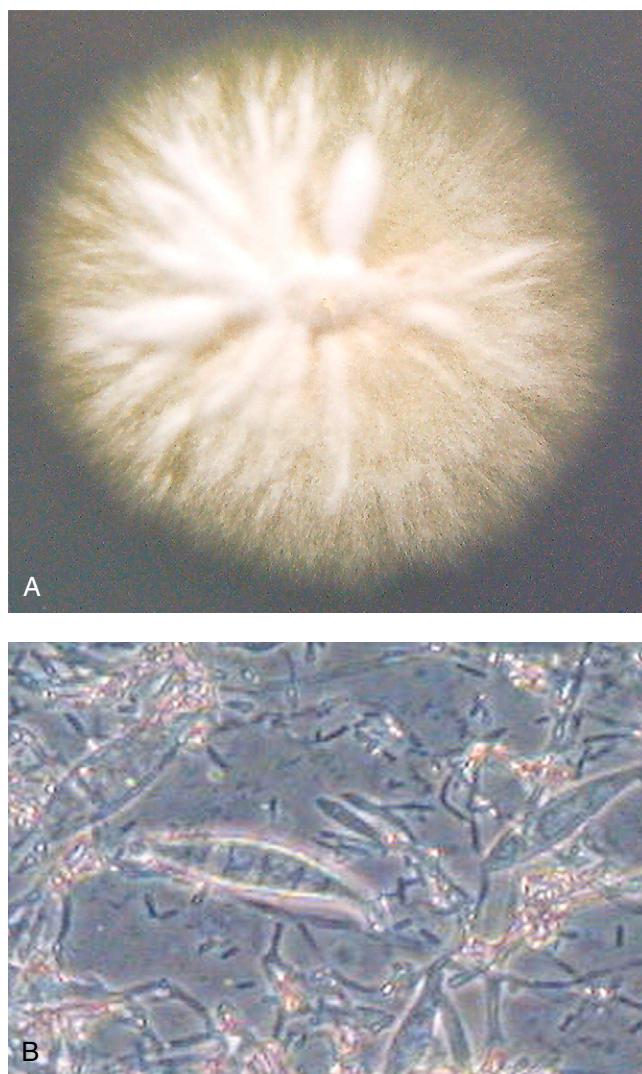




**Figure 16-6** *Epidermophyton floccosum*. (A) Colony surface. (B) Microscopic (40×). (Image courtesy of Center for Medical Mycology, Cleveland, Ohio, USA.)

dermatophyte strains and the identification of quality control (QC) strains, using the microdilution method developed at the Center for Medical Mycology.<sup>55,56</sup> Results from those studies led to adoption of an amendment to the M38A methodology, soon to be published as M38A2.<sup>57,58</sup>

The antifungal drugs used to treat dermatophytoses differ from those used against yeasts and other filamentous moulds. For instance, ciclopirox, griseofulvin and terbinafine must be included in any dermatophyte susceptibility panel. Further, the MICs of the azole class of antifungals, including fluconazole, itraconazole, posaconazole, and voriconazole, are characteristically low against dermatophytes.<sup>31</sup> Therefore, appropriate QC dermatophyte strains were needed as guidelines for susceptibility testing with these additional drugs (Table 16-3).



**Figure 16-7** *Microsporum canis*. (A) Colony surface. (B) Microscopic (40×). (Image courtesy of Center for Medical Mycology, Cleveland, Ohio, USA.)

Further modifications of the M38A microdilution method for testing of dermatophytes include the use of baby oatmeal cereal to induce conidial formation in *T. rubrum*, adjustment of inoculum size, and length of incubation.<sup>55</sup> Other methods for susceptibility testing of dermatophytes, including E strips and disk diffusion, have not yet been established.

## Treatment

The choice of treatment should be geared to the specific tinea involved. Tinea corporis and tinea cruris, for example, may be effectively cured by the use of several topical agents, many of which are now available over the counter. Tolnaftate is available in a number of vehicles, while the imidazoles econazole, ketoconazole, miconazole and clotrimazole, and the allylamine terbinafine are also very effective against dermatophytes. These agents are normally applied twice daily for at least 2 weeks, and may help decrease recurrences of infections in patients receiving oral treatment.<sup>59</sup>



**Table 16-3** Antifungal MIC ranges against QC strains (in µg/ml)

Organism	Antifungal Agent	MIC Range (µg/ml)
<i>T. mentagrophytes</i>	Ciclopirox	0.5-2.0
MRL 1957	Griseofulvin	0.12-0.5
ATCC MYA-4439	Itraconazole	0.03-0.25
	Posaconazole	0.03-0.25
	Terbinafine	0.002-0.008
	Voriconazole	0.03-0.25
<i>T. rubrum</i>	Ciclopirox	0.5-2.0
MRL 666	Fluconazole	0.5-4.0
ATCC MYA-4438	Voriconazole	0.008-0.06

The traditional antifungal agent for the treatment of tinea capitis has been griseofulvin, which is currently the only drug approved by the FDA for this application.<sup>60</sup> However, compliance with griseofulvin is generally low due to unpleasant taste and, over the years, higher doses and longer courses of treatment with this agent have been required for successful outcome.<sup>61-63</sup> The triazoles fluconazole and voriconazole, as well as allylamines such as terbinafine, are potential alternatives to griseofulvin for the treatment of tinea capitis.

Tinea unguium, or onychomycosis, continues to present a high rate of recurrence, despite low in vitro MIC values of several antifungal agents against the dermatophyte species causing nail disease. For example, primary resistance of *T. rubrum*, the major cause of onychomycosis worldwide, to terbinafine has only rarely been reported.<sup>64,65</sup> It has been suggested that host factors, including male gender, increased age, nail trauma, and poor peripheral circulation due to vascular disease or diabetes, as well as mycelial invasion into the nail itself which provides protection against antifungal activity, explain the need for long courses of treatment and high recurrence rate.<sup>64,66</sup>

Currently, oral agents such as terbinafine, itraconazole, and fluconazole, and more recently voriconazole and posaconazole, are prescribed in various treatment regimens, including intermittent, pulse, and short-duration administration. Intermittent dosing of terbinafine (350 mg daily given intermittently for three cycles of 2 weeks of treatment followed by 2 weeks off treatment) was attempted in order to increase tolerability and compliance; however, intermittent dosing did not provide a clear safety advantage and was significantly less effective than continuous dosing.<sup>67</sup> On the other hand, short-duration (3-month) treatment with terbinafine is effective, well tolerated and safe in dermatophyte onychomycosis.<sup>68</sup>

Pulse therapy with itraconazole has become standard treatment since it maintains an effective drug concentration in the nail plate.<sup>69</sup> In a recent follow-up study comparing itraconazole pulse dosing schedules, none of the patients who had complete mycologic cure at the end of the treatment period had onychomycosis recurrence after 12 or 24 months.<sup>70</sup>

Though these agents are highly effective, they may have side effects, including hepatotoxicity.<sup>71,72</sup> The nail lacquer products currently available have limited efficacy, perhaps because of their inability to penetrate to the nail bed. For example, the

mycologic cure rate for Penlac®, a topical nail lacquer containing ciclopirox, for a clinical trial in the US was 36%.<sup>73</sup> Several clinical trials for other nail lacquers with various excipients to improve penetration, as well as new oral drugs, are currently under way worldwide.

## Prevention

Tinea capitis and tinea corporis are transmitted indirectly via fallen hairs and desquamated epithelium more often than by direct bodily contact.<sup>74</sup> However, many different fomites can contribute to the transmission of dermatophyte infections, including combs, hairbrushes, and hats, while dogs and cats are major carriers of *M. canis*.<sup>75</sup> The major source of tinea pedis is the contaminated floors of homes or public locker rooms and swimming pools.<sup>74</sup> While it is assumed that nail infections, which often begin as tinea pedis, are transmitted from person to person, there is little scientific evidence supporting this contention. A recent study employing PCR-based species identification found spread of infection in all five families infected with *T. mentagrophytes* and in 20 of 31 families infected with *T. rubrum*.<sup>76</sup> In any case, prevention of the spread of dermatophyte infections depends upon the frequent disinfection of shared items in the home and of public floors where persons are likely to go barefoot. Household pets should be examined for the presence of fungal infection, and persons living within the same household as a patient infected with tinea corporis or capitis should be treated at the same time.

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# *Pneumocystis*

Michael A. Pfaller, Elias J. Anaissie

The organism *Pneumocystis* is an ascomycetous fungus that causes life-threatening pulmonary infection in debilitated and immunocompromised individuals.<sup>1</sup> Despite prophylaxis and the immunomodulating effects of highly active antiretroviral therapy (HAART), *Pneumocystis* pneumonia (PCP) remains among the most important opportunistic infections in HIV-infected patients.<sup>2-10</sup> In industrialized countries, PCP in HIV-infected patients is seen largely in those unaware of their HIV serostatus or in those non-compliant or intolerant of prophylaxis and antiretroviral therapy.<sup>2,4,6-8,11-13</sup> In sub-Saharan Africa, PCP contributes significantly to the overall death toll in the HIV-infected population, especially children.<sup>6</sup> In non-HIV associated immunosuppressed patients (transplantation, malignancy, connective tissue disease), *Pneumocystis* infection remains a significant cause of morbidity and mortality.<sup>4,6,14-19</sup>

First described in 1909, this organism was originally thought to be a protozoan parasite and was given the name *Pneumocystis carinii* in 1914.<sup>1,20-24</sup> The potential fungal nature of *Pneumocystis* was raised in the 1950s and the controversy surrounding the protozoan or fungal nature of these organisms continued into the late 20th century.<sup>21,22</sup> Despite the lack of an in vitro culture system, the application of molecular biology and other techniques has shown *Pneumocystis* to be a fungus, to be genetically diverse, host species specific, transmissible from host to host, to colonize individuals with minor degrees of immunosuppression, and to cause clinical disease by “new” (primary) infection in addition to reactivation of latent childhood acquired infection.<sup>3,5,8-12,20,22,23,25-32</sup> The species of *Pneumocystis* causing human infection is known as *Pneumocystis jiroveci* (formerly *P. carinii* f. sp. *hominis*).<sup>1,12,20,21,24</sup>

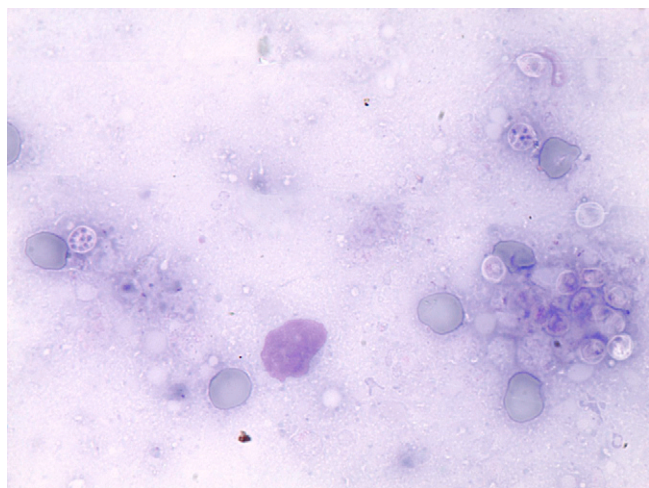
## Mycology

The history and controversy regarding the protozoan or fungal nature of *Pneumocystis* and the taxonomic decision to name the species causing infection in humans *P. jiroveci* are discussed in detail in other publications<sup>1,12,21,22,24</sup> and will not be dealt with in this chapter. Suffice it to say that in 1988, phylogenetic analyses based on the nuclear small subunit rRNA sequence alignments showed that *Pneumocystis* was a member

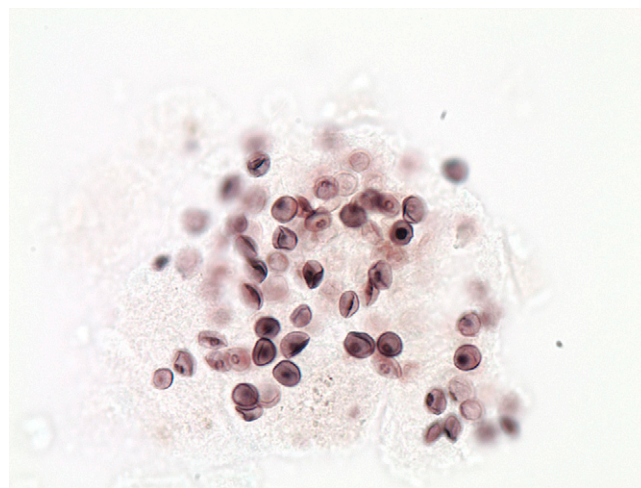
of the fungal kingdom.<sup>33,34</sup> Additional gene sequence data originating from the *Pneumocystis* Genome Project showed that the closest relative to *Pneumocystis* was the fission yeast *Schizosaccharomyces pombe*.<sup>21,22,35</sup> Based on genetic and morphologic criteria, *Pneumocystis* was placed with *S. pombe* in the Phylum Ascomycota within a newly described Class, Archiascomycetes, without designation of an Order.<sup>1,36</sup> Notably, the exact placement of *Pneumocystis* within the kingdom fungi is also controversial;<sup>1,21,22</sup> Eriksson<sup>37</sup> has opted to propose the creation of the Family Pneumocystidaceae, Order Pneumocystidales, based on gene sequence comparisons alone, and others place it at a branch point between the Ascomycota and the Basidiomycota.<sup>38,39</sup>

The morphologic characteristics of *Pneumocystis* organisms are very similar regardless of host origin.<sup>10</sup> Until 1976, *Pneumocystis carinii* was thought to represent a single zoonotic species.<sup>40</sup> It is now clear that the organism first identified as *P. carinii* is actually a family of related animals that exhibit mammalian host specificity.<sup>1,9,20,23,24</sup> Almost every mammal studied to date appears to harbor at least one species of *Pneumocystis* that is not found in any other mammal.<sup>1,9,21</sup> Among the four species that have been formally described to date, *P. carinii* and *P. wakefieldiae* infect rats,<sup>41,42</sup> *P. murina* infects mice<sup>43</sup> and *P. jiroveci* is the name given to the organisms that cause infection in humans.<sup>24,40</sup> Despite increasingly broad acceptance of the species epithet “*jiroveci*” for the agent causing human infection,<sup>20</sup> this taxonomic change is still being debated, and it remains to be seen if it will be retained by the scientific community.<sup>1,24,44,45</sup>

*Pneumocystis* has a unique tropism for the lung, where it exists as an alveolar pathogen without invading the host.<sup>3,8,10-12</sup> In rare instances (severe underlying immunosuppression or overwhelming infection), *Pneumocystis* may disseminate to various extrapulmonary sites.<sup>1,3,46-48</sup> The morphologic characteristics of *Pneumocystis* are defined using both light and electron microscopy.<sup>1,10</sup> Using light microscopy one can identify the small trophic forms (1–4 µm in diameter). The trophic forms are unicellular and ameboid in structure, with a thin wall or pellicle, and may contain two or more nuclei. In freshly prepared specimens they are ellipsoidal and often occur in clusters with other trophic forms and development stages. The trophic forms stain with Giemsa stain (Fig. 17-1) but not with



**Figure 17-1** *Pneumocystis jiroveci* in bronchoalveolar lavage fluid. Giemsa stain shows intracystic forms (magnification 1000 $\times$ ).



**Figure 17-2** *Pneumocystis jiroveci* in bronchoalveolar lavage fluid. GMS stain shows typical intact and collapsed cysts (magnification 1000 $\times$ ).

stains designed to complex with the cell wall, such as Gomori methenamine silver (GMS).<sup>1,48</sup>

The precyst, or sporocyte form, is smaller than the mature cyst (5–8  $\mu$ m vs 8  $\mu$ m in diameter, respectively) and is frequently oval in shape.<sup>1</sup> In contrast to the trophic forms, the sporocyte forms contain a rigid cell wall and may be visualized with fungal cell wall stains (Fig. 17-2). The sporocyte stage contains nuclei that are at varying stages of nuclear division (2–8 nuclei), but have not yet been compartmentalized into individual conidium structures.<sup>1</sup>

The cyst, or conidium case (ascus), is the diagnostic morphologic form of *Pneumocystis*.<sup>48</sup> It has a rigid cell wall that excludes stains such as Giemsa (see Fig. 17-1) and may be visualized with cell wall complexing stains such as GMS (see Fig. 17-2). Under light microscopy, the cyst appears as a spherical, cup-shaped or crescent-shaped object measuring approximately 8  $\mu$ m in diameter. Some cysts may appear empty or collapsed whereas others contain focal thickenings of the cyst wall that appear as dark bodies or dots in silver-stained preparations (see Fig. 17-2). The mature cyst contains eight conidia (see Fig. 17-1). The cyst wall consists of three layers as visualized by electron microscopy, and is composed of  $\beta$ -1,3-glucan, chitin, melanin and other complex polymers.<sup>10</sup> Notably, the cell membranes of the various stages do not contain ergosterol which is characteristically the membrane sterol of most fungal organisms.<sup>1,22</sup>

Elucidation of the life cycle of *Pneumocystis* has been compromised by the lack of a long-term cultivation method outside the lung.<sup>1,22</sup> Thus, the presumptive life cycle of this organism has been formulated based upon histochemical and ultrastructural studies of human- and rat-derived *Pneumocystis*.<sup>3</sup>

Although organisms may be observed within macrophages as a result of the host response to the infection, there is no evidence for an intracellular phase in the life cycle of *Pneumocystis*.<sup>1,3,8,11</sup> The trophic forms are presumed to represent the vegetative stage of the *Pneumocystis* life cycle and reproduce asexually by binary fission.<sup>1</sup> It is thought that they also participate in the sexual mode of reproduction based on identification of several fungal meiosis-specific and mating type gene homologs in genomic *P. carinii* databases.<sup>1,49</sup> Following the

mating process and nuclear fusion (karyogamy), the zygote nucleus undergoes meiosis and sporogenesis is initiated, resulting in the formation of the precyst or sporocyte. Subsequent to meiosis, an additional mitotic replication occurs, following which the nuclei and organelles are compartmentalized into eight ascogonia.<sup>1</sup> The mature spherical cyst is the end product of sporogenesis (see Fig. 17-1). Although the process of conidium release has not been described, serial ultrathin sectioning of the cyst has revealed an irregular round pore localized in the thickened area of the cyst wall (see Fig. 17-2), which is thought to be used for the release of conidia from the cyst.<sup>50</sup>

Although *Pneumocystis* DNA has been detected in environmental samples of air and water, the intact organism has never been isolated from the environment.<sup>5</sup> Indeed, it is not yet known whether *Pneumocystis* is an obligate parasite which can only propagate within its specific host or whether it is also capable of reproducing in an environmental niche.<sup>5,6,8,9</sup> A growing body of evidence demonstrating the co-evolution of *Pneumocystis* strongly suggests the absolute requirement of the host for replication of the organism, similar to other host-dependent pathogens such as *Entamoeba histolytica* or *Mycobacterium tuberculosis*.<sup>1,9,51</sup> Additional studies in humans and animal models further support a role for neonates, immunocompetent hosts and asymptomatic immunocompromised hosts as potential reservoirs, either transiently or longer term.<sup>5,6,9,12,26-28,30-32,52</sup>

## Ecology

*Pneumocystis* is ubiquitous throughout nature, infecting a variety of warm-blooded animals. Despite the potential reservoir of this fungus in rodents,<sup>22</sup> it is now understood that human pneumocystosis is not a zoonotic disease.<sup>3,9,20,21,24</sup> Molecular evidence confirms the host species-specific nature of each species of *Pneumocystis* and the genetic diversity within each species has been elicited to trace the spread of *P. jiroveci* throughout the human population.<sup>12,21,25,27-30,32,52,53</sup> Using PCR-based methods of detection and strain typing, it appears that the most important potential reservoirs of *P. jiroveci* are

children and both symptomatic and asymptomatic immunocompromised individuals.<sup>5,6,12,26-28,32,52</sup>

Evidence for widespread and early acquisition of primary *Pneumocystis* infection has long been appreciated from serology-based studies.<sup>5,6,8,9,11,12</sup> Seroprevalence studies in infants and children indicate that the percentage of antibody-positive infants increases with age; 85% seroconvert by 20 months of age.<sup>54-56</sup> More recently, *P. jiroveci* DNA was detected in approximately one-third of oral swabs obtained from infants with bronchiolitis and other mild respiratory infections.<sup>27,29,30,56</sup> Moreover, molecular typing showed that the infants harbored the same genotypes as adult patients.<sup>30</sup> These findings were taken as support for the potential role that children with primary infection might have as a reservoir of *P. jiroveci* in the community.<sup>12,27,30,56</sup>

Additional reports of the detection of *P. jiroveci* in adult populations, ranging from those without immunosuppression to those with chronic underlying diseases that have not been historically associated with its presence, suggest colonization or expansion of its host range.<sup>5,6,12</sup> It has been shown that *P. jiroveci* is carried in the respiratory tracts of asymptomatic individuals with mild immunosuppression induced by HIV or malignancy, in patients receiving long-term corticosteroid therapy for malignancy or connective tissue disorders, and in pregnant women.<sup>5,6,9,12,15,19,27-32,57</sup> The detection of *P. jiroveci* DNA in respiratory samples of these asymptomatic patient groups has been variously described as colonization, carriage, asymptomatic infection, and subclinical infection.<sup>5,6,12</sup> As with children with primary *Pneumocystis* infection, these groups of patients may be important in the human-to-human transmission of *P. jiroveci* and they may be a reservoir for future *Pneumocystis* infection in other susceptible (immunosuppressed) individuals.<sup>9,12</sup>

## Pathogenesis and pathology

Trophic forms of *Pneumocystis* adhere tightly to type I alveolar cells through interdigitation of their membranes with those of host cells.<sup>1</sup> This process is facilitated by host proteins such as fibronectin and vitronectin.<sup>3,8</sup> These host proteins bind to the surface of *Pneumocystis* and mediate attachment to integrin receptors present on the alveolar epithelium. Although binding of *Pneumocystis* organisms may induce alveolar epithelial cell growth impairment, the fungus does not alter the metabolic, structural or barrier functions of the alveolar cells.<sup>8</sup> Rather, the exuberant host inflammatory response causes injury to the alveolar epithelium with denudation of the basement membrane and subsequent hypertrophy of alveolar type II cells.<sup>3,8</sup> An increase in alveolar capillary membrane permeability occurs, leading to interstitial edema and the filling of the alveolar spaces with masses of organisms, alveolar macrophages, desquamated alveolar epithelial cells and polymorphonuclear leukocytes.<sup>3,8</sup> The resultant diffuse alveolar damage is associated with impaired gas exchange and respiratory failure.

The host immune response to *Pneumocystis* is a complex interaction between alveolar epithelial cells, CD4+ T cells, alveolar macrophages, neutrophils, and soluble mediators.<sup>58,59</sup> CD4+ T cells play a pivotal role in the host's defense by serving as memory cells for the recruitment and activation of monocytes and alveolar macrophages. The risk of *Pneumocystis*

infection increases greatly with a CD4+ T cell count of less than 200 per microliter.<sup>1,8</sup>

Alveolar macrophages are the principal phagocytes which are responsible for the uptake and degradation of the organisms within the lung.<sup>58,59</sup> The uptake of *Pneumocystis* is mediated primarily through pattern recognition receptors that interact with several components of the organism.<sup>58,59</sup> Similar to other eukaryotic microorganisms, antigenic variation involving the major surface glycoprotein of *Pneumocystis* provides a mechanism allowing the fungus to manipulate and evade the innate immune response.<sup>58-60</sup>

Effective inflammatory responses in the host are necessary to control PCP, although overactive inflammation promotes lung injury.<sup>3,8,59</sup> Severe PCP is characterized by CD8+ T cell neutrophilic lung inflammation, resulting in alveolar damage, impaired gas exchange and respiratory failure.<sup>8</sup> Indeed, respiratory impairment and death are more closely related to the degrees of lung inflammation than to the organism burden.<sup>8,11</sup>

Pathologically, the lungs in PCP are firm and enlarged. Diffuse pneumocystosis affects all lobes of the lungs and histologically shows interstitial thickening of the alveolar septa and frothy honeycombed material in the alveoli.<sup>1</sup> Organisms appear to be more numerous, and the associated inflammation significantly less, in patients with AIDS than in other immunocompromised hosts.<sup>11</sup>

Other less common pathologic characteristics may be seen in individuals with AIDS.<sup>8,11</sup> Diffuse alveolar damage may predominate without alveolar exudates. Cystic and cavitary lesions, primarily in the upper lobes, may develop and are often complicated by pneumothorax resulting from rupture of the cysts into the pleural space.<sup>3,11</sup> Single nodules and non-caseating granulomas, sometimes with calcification, have been infrequently described. Extrapulmonary pneumocystosis has been reported involving almost all organs and tissues.<sup>46,47</sup> Dissemination appears to be by both hematogenous and lymphatic routes. The most common sites of dissemination are the lymph nodes, spleen, liver, and bone marrow.<sup>46,47</sup> Lesions consist of masses of organisms enclosed in eosinophilic, foamy, focally calcified material.<sup>61</sup>

## Epidemiology

### Transmission

PCP has been diagnosed in patients worldwide. Studies in humans and animals clearly favor airborne transmission and there is now compelling evidence for both human and environmental sources of infection.<sup>3,5,6,11,12,16-18,25-30,32</sup> Traditionally it was thought that pneumocystosis resulted from reactivation of latent infection that was acquired during childhood.<sup>1,9,12,29</sup> Although primary infection clearly does occur early in life,<sup>5,6</sup> there is now evidence that *Pneumocystis* organisms are frequently acquired and cleared by the immune system of immune competent humans, that patients with recurrent episodes of PCP are often reinfecting with a different genotype of *P. jiroveci* associated with each new episode of infection, and that allelic variation patterns in isolates of *P. jiroveci* are correlated with the patient's place of diagnosis and not their place of birth.<sup>5,6,11,12,25,28,29,52</sup> Thus, reactivation of latent infection may certainly occur but the bulk of the evidence supports the



acquisition of new infection via environmental exposure, or more likely person-to-person transmission.<sup>9,12,25,52</sup>

Evidence of early infection comes largely from seroprevalence surveys conducted in infants and children.<sup>54,55,62,63</sup> Likewise, the high rate of PCP in the first 6 months of life among HIV-infected infants supports the ubiquitous presence of *P. jiroveci* in the environment.<sup>6</sup>

Primary infection with *Pneumocystis* in immunocompetent individuals is generally thought to be asymptomatic or associated with mild non-specific symptoms.<sup>3,12,26-29</sup> *Pneumocystis* DNA has been detected in nasopharyngeal aspirates of healthy immunocompetent children and in immunocompetent infants with mild acute upper respiratory tract infections.<sup>12,27,29,30</sup> Evidence of mild PCP was observed histologically in 32% of 161 autopsy specimens from a study of sudden infant death syndrome.<sup>64</sup> These findings have raised the possibility that healthy children may constitute an important natural reservoir of *P. jiroveci* organisms and play a role in the circulation and transmission of *P. jiroveci* in the community.<sup>5,12</sup>

Several studies have employed PCR-based molecular methods to detect *P. jiroveci* DNA in respiratory samples of both HIV-positive individuals and non-immunocompromised hosts with no clinical evidence of PCP.<sup>25,28,30-32,52,53</sup> Among HIV-positive individuals without evidence of PCP, estimates of the carriage rate range from 9% to 69%.<sup>12,32</sup> High rates of carriage have been documented in HIV-negative adults with varying degrees of chronic pulmonary disease and one study demonstrated the presence of *Pneumocystis* DNA among 20% of otherwise healthy adults.<sup>5,6,12,28,30,31,53</sup> Whereas these studies may support the hypothesis that *P. jiroveci* can exist in its host for long periods of time without causing clinical disease, they also may be interpreted to show that *P. jiroveci* is ubiquitous in the environment and that exposure in humans is characterized by intermittent colonization or subclinical infection.<sup>5,6,9,12</sup> Further evidence against the theory of reactivation of latent infection as the sole mechanism of clinically evident PCP includes findings that humans and animals clear *Pneumocystis* after infection, the presence of genotype switching in chronic carriers and in repeat episodes of PCP, and geographic variability in the distribution of *Pneumocystis* strains and infection with *Pneumocystis*.<sup>9,11,12,25,28,32</sup>

Airborne transmission of *Pneumocystis* has clearly been demonstrated in animal models of infection.<sup>26,65</sup> In one study,<sup>26</sup> immunocompetent mice were shown to become carriers following exposure to *Pneumocystis*-infected severe combined immunodeficiency (SCID) mice. These carriers were then shown to be capable of transmitting the organisms to both previously uninfected SCID mice and to a second group of immunocompetent mice.<sup>26</sup>

*P. jiroveci* DNA has been detected in environmental samples of air and pond water but not in soil.<sup>5,66-68</sup> Air filters placed in hospital rooms and attached to ventilators of patients with PCP have also been found to contain detectable *Pneumocystis* DNA.<sup>69-71</sup> The presence of *Pneumocystis* in the air is consistent with an environmental route of infection but could also arise directly from colonized or infected individuals.<sup>5</sup>

Clusters of PCP in oncology and transplant centers suggest the possibility that *P. jiroveci* can be transmitted from person to person.<sup>5,9,12</sup> Epidemiologic investigations of such clusters have demonstrated that many of the infected patients had contact with each other.<sup>14,16-18</sup> Whereas this may be constructed

as evidence for patient-to-patient transmission, it could also represent a common environmental exposure.<sup>5</sup>

Genotypic analysis of *P. jiroveci* isolates from several different epidemiologically linked clusters of infection have shown that some, but not all, of the infected patients within a given cluster shared a common genotype.<sup>14,16-18,30</sup> Although the finding of the same genotype of *P. jiroveci* in multiple infected patients supports the potential for person-to-person transmission, the fact that other patients in the same cluster of infections did not share the “epidemic” strain indicates that the direct person-to-person spread may not be the only, or even the most important, mode of transmission.<sup>3,5,9,11,12,20,24,25,28,29</sup>

Studies of mutations in the *Pneumocystis* dihydropteroate synthase (DHPS) gene have also shed some light on transmission of *P. jiroveci*.<sup>9,11,12,25,72,73</sup> DHPS mutations are seen in organisms from patients who have been treated with DHPS inhibitors such as sulfamethoxazole or dapsone.<sup>9,11,25</sup> Huang et al<sup>74</sup> documented mutant DHPS genotypes in patients with PCP who were newly diagnosed with HIV and who had not received treatment or prophylaxis for PCP. In such patients one would expect to find the wild-type sequence at the DHPS locus; however, more than half of the patients were infected with strains of *P. jiroveci* with DHPS mutations.<sup>72,74</sup> These findings suggest that the organism was acquired from an individual with prior drug exposure, either directly or through an intermediate (environmental) source.<sup>1,11,72</sup>

Finally, in an effort to document nosocomial transmission of *Pneumocystis*, it has been shown that some asymptomatic healthcare workers may be *Pneumocystis* carriers.<sup>12,75-77</sup> Healthcare workers who were in contact with HIV patients were shown to be at increased risk for carriage of *Pneumocystis* (24%) compared with non-exposed workers (11%), suggesting patient-to-healthcare worker transmission.<sup>12,75</sup> These findings were complicated by the fact that not all of the colonized healthcare workers carried the same strains found in local PCP patients during the same time period.<sup>75</sup> Although this and other studies<sup>75-77</sup> suggest that transmission from PCP patients to carriers can occur, other sources of exposure must exist.<sup>12</sup>

## Incidence and risk factors

The first cases of PCP were reported in malnourished children in Europe during World War II.<sup>1</sup> In the late 1960s and early 1970s, there were fewer than 100 cases per year of PCP in the United States.<sup>11,78</sup> The disease was recognized in patients who were immunocompromised because of malignancies, immunosuppressive therapy or congenital immunodeficiencies. Solid organ transplantation increased the number of patients at risk for PCP although rates decreased after the introduction of chemoprophylaxis. Without chemoprophylaxis, rates of PCP are 5–25% in transplant patients, 2–6% in patients with collagen vascular disease, and 1–25% in patients with cancer.<sup>11</sup> Defects in CD4+ T lymphocytes are a primary risk factor for developing PCP, but defects in B cells and antibody production may also predispose to PCP.<sup>1,2,6,11,15,19,59</sup> Therapy with monoclonal antibodies such as adalimumab, infliximab, etanercept, and rituximab has been recently associated with development of PCP.<sup>78a-78f</sup>

Following the onset of the AIDS epidemic in the early 1980s, there was a marked increase in the incidence of cases of PCP reported to the CDC that peaked in 1990 at about 20,000

cases per year.<sup>11</sup> Clusters of PCP cases in homosexual men and intravenous drug users were one of the first indicators of the HIV epidemic and PCP rapidly became the leading AIDS-defining diagnosis in HIV-infected individuals.<sup>8,11</sup> In the early stages of the epidemic, PCP rates were as high as 20 per 100 person-years for those with CD4+ counts <200 cells/ $\mu$ l.<sup>6,79</sup> PCP was responsible for 66% of AIDS-defining illnesses, and an estimated 75% of HIV-infected patients would develop PCP during their lifetime.<sup>6,11</sup>

In the early 1990s, there was a decline in incidence that was largely due to the widespread use of PCP prophylaxis.<sup>6</sup> Although the absolute numbers of cases remained stable from 1989 to 1992 due to the increasing incidence of AIDS, the percentage of AIDS cases with PCP declined from 53% in 1989 to 49%, 46%, and 42% in 1990, 1991, and 1992, respectively.<sup>6</sup>

Data from the Adult and Adolescent Spectrum of HIV Disease (ASD) Project indicated a marked reduction in the incidence of PCP, and other opportunistic infections, in 1996 and 1997, when HAART first became widely available.<sup>1,6,11,80</sup> PCP cases declined by 3.4% per year from 1992 through 1995, whereas the rate of decline increased to 21.57% per year from 1996 through 1998.<sup>6,80</sup> Despite this improvement, PCP remains the most common AIDS-defining opportunistic infection in the US.<sup>1,6,11</sup>

Among HIV-infected adults in the US who developed PCP from 1999 through 2001, almost 44% of cases occurred in patients not receiving medical care, most of whom were likely not known to be HIV positive.<sup>6,13</sup> An additional 41% of patients were prescribed prophylaxis but either did not adhere to treatment or developed PCP despite taking their medications appropriately.<sup>6</sup> Prophylaxis was not prescribed in 9.6% of patients who were under medical care and should have received prophylaxis based on current recommendations.<sup>6</sup> More recently, Teshale et al<sup>13</sup> confirmed that the proportion of eligible persons receiving primary prophylaxis remains unacceptably low. They found that persons with fewer treatment visits or those who are injection drug users, women or Hispanic were significantly less likely to receive PCP prophylaxis.

A CD4+ cell count <200 cells/ $\mu$ l remains an important risk factor for PCP in adults and in children greater than 6 years of age.<sup>6,11</sup> The occurrence of PCP in infants is not related to the absolute CD4+ cell count in the same manner as in adults, although it is related to the percentage of CD4+ cells and CD4+ cell counts are below normal in children less than 1 year of age with PCP.<sup>6,81</sup> Other clinical factors such as sex, race or ethnicity, and HIV transmission category have been examined as risk factors for PCP, without compelling evidence to indicate a change in risk.<sup>6,11</sup> Among patients with malignancies and transplantation, risk factors include prolonged corticosteroid administration, other immunosuppressive regimens, CD4+ count <200 cells/ $\mu$ l, chronic graft-versus-host disease (GvHD), and relapse of hematologic malignancy.<sup>6,11,15,19</sup>

## Clinical manifestations

*Pneumocystis jiroveci* causes clinically apparent pneumonia virtually exclusively in immunosuppressed patients.<sup>8</sup> Patients with AIDS often have a more insidious progression to clinical disease than other immunosuppressed individuals.<sup>8,10,11</sup>

The clinical presentation is characterized by persistent non-productive cough (although sputum production may occasionally be seen), fever with sweats, chest tightness and progressive shortness of breath.<sup>8,11</sup> Hemoptysis may also be present. A small proportion of patients may remain asymptomatic. *P. jiroveci* infection is not only confined to the lungs but may be disseminated via lymphatic and hematogenous routes in 1–3% of patients.<sup>1,3,46,47,61</sup> This is often associated with the administration of aerosolized pentamidine prophylaxis due to the lack of activity outside the lung.<sup>3</sup> Dissemination most frequently involves the lymph nodes (44%) followed by the spleen, bone marrow, and liver.<sup>1,3,10</sup> Other less common sites of extrapulmonary involvement include the adrenal glands, thyroid, gastrointestinal and genitourinary tracts, pancreas, ear, eyes, and skin.<sup>46,47,61,82</sup> Infection of multiple extrapulmonary sites has been associated with a rapidly fatal outcome.<sup>1,61</sup>

## Diagnosis

### Physical examination

Physical examination reveals fever and tachypnea, tachycardia, cyanosis, and fine basilar crackles and rhonchi on auscultation, although chest examination may be normal in a significant proportion of patients (up to 50% among HIV-infected patients). Extrapulmonary pneumocystosis, such as pleural effusions, skin lesions or hepatosplenomegaly, may be seen in patients receiving aerosolized pentamidine prophylaxis.<sup>1,3,46,47,61,82</sup> Clinical findings are reflective of the site or organ involved, such as retinal cotton wool spots in ocular involvement or pancytopenia in patients with bone marrow involvement.<sup>1</sup>

PCP in children can be associated with cyanosis, nasal flaring, a mild cough without fever and, in severe cases, intercostal retractions. Impaired oxygen diffusion capacity, alterations in lung compliance, total lung capacity and vital capacity result in hypoxemia.<sup>1</sup>

### Diagnostic studies

Screening tests should be performed when PCP is suspected clinically, and include assessment of oxygenation at rest and after exercise and pulmonary function tests and serum lactic dehydrogenase (LDH). Indeed, PCP is unlikely in the presence of an increase in oxygen saturation with exercise,<sup>83</sup> a diffusing capacity for carbon monoxide (DLCO) >70% of predicted<sup>84</sup> or a normal LDH.<sup>85,86</sup> The prognostic significance of LDH is particularly important; the higher the LDH, the lower the likelihood of survival, and increasing LDH values despite optimal therapy is predictive of a poor outcome.<sup>86</sup>

Arterial blood gas measurement is mandatory in order to determine the extent of respiratory failure. The oxygenation impairment induced by pneumocystosis can be detected by a widening of the alveolar–arterial oxygen gradient ((A-a) DO<sub>2</sub>) correlated with the severity of disease and by respiratory alkalosis.<sup>1</sup> It should be noted, however, that a significant number of patients can have a normal (A-a) DO<sub>2</sub> gradient at rest.

Chest x-ray (CXR) most often shows diffuse bilateral interstitial or alveolar infiltrates, while lobar or segmental consolidation, cysts, nodules and cavities, pneumothoraces and pleural effusions are less common.<sup>1</sup> Administration of

aerosolized pentamidine has been associated with an increase in the frequency of apical infiltrates and pneumothoraces.<sup>11</sup> The severity of abnormalities on CXR is considered prognostic and can be correlated with a higher mortality.<sup>1</sup>

High-resolution computed tomography (HRCT) is more sensitive than CXR, particularly among HIV-positive patients, and usually reveals patchy or nodular ground-glass attenuation;<sup>87-89</sup> a negative HRCT practically excludes the diagnosis of PCP.

Gallium-67 citrate scan is highly sensitive but non-specific in patients with PCP, and usually shows diffuse bilateral uptake.<sup>90</sup> Because of its cost and the 2-day delay in obtaining results, the test is usually limited to patients with normal CXR but suspected of having PCP although HRCT of the chest has rendered the Gallium-67 test almost obsolete.

## Laboratory diagnosis (Table 17-1)

The diagnosis of *P. jiroveci* infection is almost entirely based upon microscopic examination of clinical material including induced sputum, bronchoalveolar lavage (BAL) fluid, bronchial brushings, and transbronchial or open lung biopsy specimens.<sup>1</sup>

## Sputum induction

Although examination of induced sputum is less invasive and may be useful in AIDS patients with a high organism load, it has a 20–25% false-negative rate and is not useful in HIV-negative immunocompromised hosts.<sup>1,11</sup> Even in HIV-positive patients, the sensitivity of this method is highly variable, ranging from 55% to 92%.<sup>91,92</sup> Factors that reduce the test sensitivity include the prevalence of PCP in the patient population, use of PCP prophylaxis (particularly aerosolized pentamidine),<sup>93</sup> and local expertise with the test (sputum induction and laboratory processing and interpretation of the specimen). The occurrence of a recent acute PCP infection lowers the specificity of positive induced sputum because visible organisms may persist in a large proportion of patients even following successful therapy.<sup>94</sup>

## Bronchoalveolar lavage and other diagnostic methods

Examination of BAL fluid has been shown to have a sensitivity of 90–100% and in most instances alleviates the need for transbronchial or open lung biopsy<sup>11,48</sup> although one

**Table 17-1** Performance of diagnostic tests for Pneumocystosis

Method	Specimen	Sensitivity	Specificity	Comments
<b>Conventional stains</b>				
Calcofluor white	BAL	~75%	≥99%	Lower sensitivity with low organism burden*
Gomori methenamine silver	BAL	~75%	≥99%	Lower sensitivity with low organism burden*
Diff-Quick	BAL	<50%	≥99%	Lowest sensitivity. Not recommended.
<b>Immunofluorescent antibody</b>	BAL	~90%	~ 95%	Better sensitivity but lower specificity than conventional methods. If positive result, confirm with another method.
<b>Real-time PCR</b>	BAL/ OPW/NPW	≥99%	≥99%	Real-time PCR superior to conventional PCR techniques. Lower sensitivity with low organism burden* Potential for false positive results.
<b>Reverse transcriptase PCR</b>	BAL/IS/OPW	≥99%	≥85%	Identification of messenger RNA suggests viable organisms. Lower diagnostic performance if used on IS and OPW
<b>β-glucan</b>	Serum	≥ 90%	≥85%	Non-invasive, sensitive test which carries prognostic significance and may be used to monitor response to therapy

Key: BAL: bronchoalveolar; IS: induced sputum; OPW: oropharyngeal wash; NPW: nasopharyngeal wash;

\*Lower sensitivity with low organism burden: e.g. aerosolized pentamidine, non-HIV immunocompromised patient



study suggested a much lower sensitivity (62%) with aerosolized pentamidine prophylaxis.<sup>95</sup> Transbronchial biopsy is almost always diagnostic in such patients. If focal infiltrates are present, sampling the most heavily involved lobes on chest radiograph can increase the diagnostic yield.<sup>96</sup> BAL fluid can also be obtained by a BAL catheter.<sup>97</sup> Alternatively, endotracheal aspirates obtained from intubated patients appear to have high sensitivity and may obviate the need for bronchoscopy.<sup>98</sup>

The 30% rate of pneumothorax with transthoracic needle biopsy and the cost and invasiveness of lung biopsy (by thoracotomy or by video-assisted thoracoscopic surgery) limit the usefulness of these methods despite their very high diagnostic yield.

A variety of stains have been used to detect *P. jiroveci* including Giemsa, Giemsa-like, toluidine blue, GMS, periodic acid-Schiff, Gram-Weigert, Calcofluor, and immunofluorescence<sup>1,11,48</sup> (also see Chapters 4 and 5 in this book). Giemsa and Giemsa-like (e.g., Wrights, Diff Quick) stains demonstrate the trophic forms and do not stain the cyst wall (see Fig. 17-1), whereas GMS stains the cyst wall but not the trophic forms (see Fig. 17-2). Immunofluorescent techniques stain both trophic forms and the cyst walls (Fig. 17-3). Monoclonal antibody-based immunofluorescent methods have been shown to be more sensitive than conventional colorimetric stains such as GMS and Giemsa (90.8% vs 79.4% and 49.2%, respectively) but are less specific (81.9% vs 99.2–99.6%, respectively).<sup>48</sup> It is suggested that if an immunofluorescent method is used as the primary staining method in the clinical microbiology laboratory, then confirmatory staining with a second method should be performed to increase the specificity and positive predictive value of the final test result.<sup>48</sup>

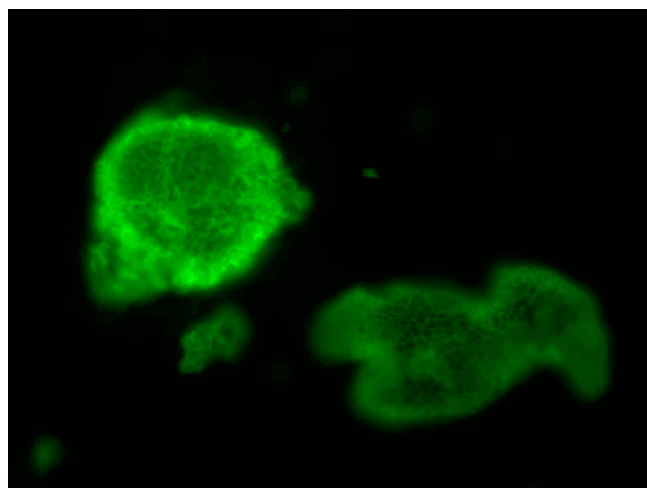
Serologic assays to detect antibodies specific for *P. jiroveci* have been useful for epidemiologic studies, but not for diagnosis of PCP.<sup>1</sup> Likewise, antigen capture assays have not proven useful.

PCR-based detection of *P. jiroveci* DNA has been shown to have greater sensitivity and specificity for the diagnosis of

PCP from respiratory specimens than conventional (Giemsa or GMS) microscopic methods.<sup>99,100</sup> The greater sensitivity of PCR-based methods also may allow diagnostic testing to be performed on upper respiratory tract specimens, where the number of *P. jiroveci* organisms is low.<sup>100</sup> Oropharyngeal washes and nasopharyngeal aspirates can be carried out on pediatric patients who are unable to sustain a more invasive procedure. Both types of specimens have been successfully used for the diagnosis of PCP using PCR.<sup>100</sup>

Although the detection of *P. jiroveci* DNA in respiratory tract samples has proven to be very successful and of high diagnostic value for PCP,<sup>100,101</sup> at present it remains largely restricted to research laboratories, and is not often used in routine clinical diagnostic laboratories.<sup>102</sup> PCR-based methods can be more labor intensive, expensive and time consuming than conventional diagnostic methods and remain essentially non-standardized.<sup>11,52</sup> No uniform target gene, PCR technique or strain nomenclature has emerged from the multiple research groups that are studying *Pneumocystis* and so there is a lack of consensus regarding the optimal approach to the diagnosis of PCP using this technology.<sup>52</sup> Furthermore, in patients with positive PCR results in sputum or BAL fluid but with negative microscopy, clinical management remains a challenge because the presence of *Pneumocystis* DNA does not necessarily indicate viable organisms or infectivity.<sup>11,101</sup>

Presently, PCR-based methods are most useful in genotyping *P. jiroveci*.<sup>16,25,29,52,72,73</sup> Molecular techniques using DNA sequence polymorphisms have been useful in epidemiologic studies of colonization and transmission and have shown that some patients may harbor more than one strain of *P. jiroveci*.<sup>9,12,20,24,25</sup> Likewise, the emerging resistance to trimethoprim-sulfamethoxazole is being investigated with the use of molecular techniques to study mutations in the DHPS gene which encodes the target enzyme for sulfamethoxazole and dapsone.<sup>9,11,30,72,73</sup> Based on evidence from other microorganisms, it has been hypothesized that certain DHPS mutations may affect substrate (sulfonamide) binding to the target enzyme in *P. jiroveci* and may be associated with drug resistance.<sup>9,72,73</sup> Indeed, several reports have implicated specific mutations in *P. jiroveci* DHPS genes that are associated with the failure of prophylaxis and treatment and an increased risk of death.<sup>72,73</sup> Furthermore, these mutations may also be selected for by exposure of patients to sulfa-containing drugs.<sup>72</sup> The importance of these findings is tempered by the fact that most patients harboring *P. jiroveci* that contains DHPS mutations still have a response to trimethoprim-sulfamethoxazole.<sup>9,11,72,73</sup> Recent data suggest that serum  $\beta$ -D-glucan, which is the major component of the cell wall of *Pneumocystis*, has excellent performance in the diagnosis of PCP and may also carry prognostic significance.<sup>102a–102g</sup> Preemptive PCP therapy may even be possible with serial monitoring of serum  $\beta$ -D-glucan values.<sup>102h</sup>



**Figure 17-3** *Pneumocystis jiroveci* in bronchoalveolar lavage fluid. Immunofluorescent technique stains both trophic forms and cyst wall (magnification 1000 $\times$ ).

## Prevention (Table 17-2)

Prevention of PCP is a logical strategy to reduce the morbidity and mortality that *P. jiroveci* can produce in susceptible individuals. The approaches that may be considered to prevent PCP include reduction of exposure to the organism, enhanced immunocompetence, and chemoprophylaxis.<sup>1,3,5,6,8–15</sup>

**Table 17-2** Pneumocystis pneumonia (PCP) prophylaxis

Regimen	Adverse reactions	Comments, special populations
<b>Regimen of choice</b>		
TMP-SMX, PO 1 DS tablet three times a week or 1 SS tablet daily	<p>Fever, rash, GI upset, neutropenia, transaminitis, hyperkalemia.</p> <p>Every attempt should be made to continue TMP-SMX before switching to alternative regimen.</p> <p><b>If allergic to TMP-SMX:</b> attempt desensitization</p> <p><b>If minor allergic reactions:</b> treat with antihistamines and continue TMP-SMX if clinically feasible.</p> <p><b>If significant but non-life-threatening reaction:</b> hold TMP-SMX until the adverse reaction has resolved then resume TMP-SMX in a dose escalation fashion (rather than at full dose).</p>	<p>Risk of PCP on TMP-SMX: &lt; 2.5 %.</p> <p>Prevents other infections ♦</p> <p><b>In cancer patients:</b> Start TMP-SMX prophylaxis prior to chemotherapy; hold during chemotherapy and restart when ANC &gt; 1500/<math>\mu</math>L off growth factor and platelets &gt; 75,000/<math>\mu</math>L. Give folic acid 1 mg PO QD. If unable to swallow pills, use pediatric suspension [5 cc suspension contains 40 mg trimethoprim, 200 mg sulfamethoxazole]</p> <p><b>In methotrexate recipients:</b> (rheumatic disease, allogeneic HSCT, other): the combined use of TMP-SMX and methotrexate may cause bone marrow suppression because both methotrexate and trimethoprim, are folate antagonists. Hence, TMP-SMX dose should not exceed one DS tablet three times a week or one SS tablet daily.</p>
<b>Alternative</b>		
Dapsone, PO 100 mg daily or 50 mg twice daily	<p><b>Dapsone adverse reactions:</b></p> <p>Fever, rash, GI upset, hemolytic anemia, methemoglobinemia. Screen patients for G 6PD deficiency before starting dapsone. Discontinue if methemoglobinemia &gt; 10 %.</p> <p>Cross-reactivity with TMP-SMX (rash) but well tolerated even after rash to TMP-SMX: do not give concurrently with TMP-SMX or during desensitization.</p>	Risk of PCP on dapsone: 15 %.
<p>Dapsone, PO, 50 mg daily plus <i>one weekly dose</i></p> <p>Pyrimethamine 50 mg</p> <p>Leucovorin 25 mg</p> <p>OR <i>one weekly dose</i></p> <p>Dapsone 200 mg plus</p> <p>Pyrimethamine 75 mg plus</p> <p>Leucovorin 25 mg</p>	<p><b>Pyrimethamine adverse reactions:</b></p> <p>Rash, GI upset, neutropenia, anemia, thrombocytopenia.</p> <p>Rarely: headache, insomnia, seizures.</p> <p>Use folinic acid useful to reduce bone marrow suppression</p>	For adequate prophylaxis against toxoplasmosis, add pyrimethamine and leucovorin to dapsone

**Table 17-2** Pneumocystis pneumonia (PCP) prophylaxis—cont'd

Regimen	Adverse reactions	Comments, special populations
Aerosolized pentamidine (AP) 300 mg monthly (via Respigard II nebulizer)	Cough. Atypical presentation while on AP: PCP may be confined to the upper lobes of the lungs or present with extrapulmonary sites of infection.	Risk of PCP on AP: 15 % <b>In cancer patients</b> at high risk for PCP: Give 300 mg dose every 2 weeks for 1 month then monthly. <b>In allogeneic HSCT:</b> continue 300 mg dose every 2 weeks (higher risk of PCP breakthrough) <b>In HIV positive patients:</b> Avoid AP if CD4 counts < 100/ $\mu$ L (less effective)
Atovaquone suspension, PO 750 mg twice daily	Fever, rash, pruritis, GI upset, transaminase elevation, headache, insomnia, dizziness. Rarely, anemia, neutropenia.	Better bioavailability of suspension (50 % vs tablet 25%). Take atovaquone with food. Use tablets three times a day. For adequate prophylaxis against toxoplasmosis, add pyrimethamine and leucovorin.

Key: PO; oral; GI: gastrointestinal; HSCT: hematopoietic stem cell transplant recipient; ANC: absolute neutrophil count. TMP-SMX: trimethoprim-sulfamethoxazole; DS: double strength; 160 mg of trimethoprim plus 800 mg of sulfamethoxazole; SS: single strength; 80 mg of trimethoprim plus 400 mg of sulfamethoxazole;

◆Prevents other infections including those caused by *Toxoplasma gondii*, *Isospora*, *Listeria*, *Nocardia*, *Salmonella*, *Legionella*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and many gram-negative bacilli. If the risk of toxoplasmosis is elevated, use one DS tablet TMP-SMX daily.

## Reduction of exposure to the organism

Air sampling studies and animal models of infection indicate that the route of transmission of *Pneumocystis* is by air.<sup>5,9,12,26</sup> Multiple human studies have shown that *P. jiroveci* DNA can be recovered from air samples taken from patient rooms.<sup>5</sup> Often, but not always, the *P. jiroveci* genotype in the air matches that of the patient in the room.<sup>70,103</sup> Transmission of *P. jiroveci* from patients with active PCP to susceptible persons is suggested by numerous reports of clusters of PCP cases, as well as by the demonstration of transmission of *Pneumocystis* in animal models.<sup>5,14,16-18,26,30,32</sup> Three recent clusters of PCP in renal transplant patients provide epidemiologic and molecular evidence that limited nosocomial transmission of *P. jiroveci* can occur among severely immunocompromised patients, although it is unclear whether this clustering starts with an index patient, comes from a temporarily increased reservoir of carriers in the population, or comes from an environmental source.<sup>14,16,18</sup>

Although these data may not be sufficient to support a policy of isolation of patients with suspected PCP at the time of entry to the hospital or clinic, avoiding contact between severely immunocompromised patients at risk for PCP and patients with active PCP does seem warranted<sup>104</sup> and avoiding

room sharing, particularly early in the treatment of PCP, seems prudent.

## Enhanced immunocompetence

Prevention of PCP through enhanced immunocompetence is commonplace.<sup>1,8,11</sup> The remarkable decrease in the incidence of PCP among HIV-infected persons since the introduction of HAART therapy is clear evidence of the usefulness of this approach.<sup>11</sup> Likewise, management of transplant recipients and cancer patients seeks to minimize the period and severity of immune deficiency by minimizing chemotherapy.<sup>8,15,19</sup>

## Chemoprophylaxis

Clearly the most widely used and successful strategy for preventing PCP is to use specific chemoprophylaxis in patients during periods of greatest susceptibility for infection.<sup>6,8,11,13</sup>

### Primary prophylaxis

Primary prophylaxis in HIV-infected adults, including pregnant women and patients receiving HAART, should begin when the CD4+ cell count is <200 cells/ $\mu$ L or if there is a history of oropharyngeal candidiasis.<sup>13,105,106</sup> Prophylaxis should also be given to patients not receiving HAART and to those



receiving HAART who have a CD4 count <200 cells/μl or who develop PCP at a higher CD4 count.

Among HIV-negative patients, prophylaxis should be considered among patients with congenital immunodeficiencies and those receiving immunosuppressive therapy, including patients with leukemia and lymphoma, hematopoietic and solid organ transplant recipients and patients with connective tissue disease that are treated with corticosteroids and/or other immunosuppressive agents.<sup>6,11</sup>

Trimethoprim-sulfamethoxazole (TMP-SMX) is the drug of first choice for prophylaxis<sup>8,11</sup> and can be given either as a daily dose (one single or double-strength tablet)<sup>107</sup> or one double-strength tablet three times weekly.<sup>108</sup> Although less well tolerated, the daily double-strength tablet dose of TMP-SMX may offer better protection against common respiratory bacterial infections<sup>109,110</sup> and against toxoplasmosis.<sup>111</sup> Desensitization to TMP-SMX should be considered for patients who develop significant adverse events such as fever and rash.<sup>112,113</sup> Alternatively, TMP-SMX may be reintroduced at a reduced dose or frequency.

In those patients unable to tolerate TMP-SMX, dapsone 100 mg daily (or 50 mg twice daily) is the preferred regimen with addition of pyrimethamine for patients who are seropositive for toxoplasma and have a CD4 count <100 cells/μl. Aerosolized pentamidine 300 mg monthly is a good alternative for patients who cannot tolerate TMP-SMX or dapsone provided CD4 count is >100 cells/μl. More frequent dosing (every 2 weeks) may be needed for patients whose CD4 count is <100 cells/μl. Administration of aerosolized pentamidine has been suspected of promoting atypical pulmonary and/or extrapulmonary *Pneumocystis* infections.<sup>3,11,114</sup> Atovaquone at a dose of 1500 mg daily has also been shown effective in HIV-positive and HIV-negative patients.

**Special considerations** Children born to HIV-infected mothers should receive TMP-SMX prophylaxis starting at age 4–6 weeks.<sup>115</sup> Prophylaxis should be discontinued if children are later shown not to be infected with HIV. By contrast, prophylaxis should continue for the first year of life and even longer, depending on age-specific CD4+ T lymphocyte count thresholds. Lifelong prophylaxis is required for children with a history of PCP.<sup>115</sup>

Systemic chemoprophylaxis for PCP should also be given to pregnant women, preferably starting during the second trimester (to avoid potential teratogenicity during the first trimester) and consists of TMP-SMX with dapsone as an alternative. Aerosolized pentamidine can be safely given during the first trimester.

### Secondary chemoprophylaxis

Patients who have had previous episodes of PCP should receive life-long secondary prophylaxis unless immune reconstitution has been achieved as a result of HAART<sup>13</sup> or because immunosuppressive therapies have been discontinued.

### Discontinuation of prophylaxis

Primary or secondary prophylaxis should be discontinued in HIV-infected patients who have had response to HAART as shown by an increase in CD4+ cell count to greater than 200 cells/μl for a period of 3 months.<sup>11,13</sup> Likewise, prophylaxis should be reinstituted if the CD4+ cell count later falls to <200 cells/μl or if PCP occurs at a higher CD4 count.

## Therapy (Table 17-3 and Fig. 17-4)

The drug of choice for the treatment of PCP remains TMP-SMX, which is also the mainstay for primary and secondary prophylaxis.<sup>8</sup> Oral TMP 320 mg plus SMX 1600 mg (two double-strength tablets) every 8 hours should be given for 21 days for HIV-positive patients. A 14 day course of therapy is appropriate in other patient populations.

TMP-SMX is often associated with adverse reactions, especially in AIDS patients and following solid organ or blood and marrow transplantation. Common side effects include rash and fever, gastrointestinal intolerance, elevated liver function tests (stop treatment if transaminases ≥3–5 times normal values and associated with symptoms of hepatitis). Neutropenia may occur particularly in patients on other myelosuppressive regimens but may respond to granulocyte colony-stimulating factor. Hyperkalemia may also be seen. About 11% of patients receiving oral TMP-SMX experience recrudescence of PCP.<sup>8</sup>

Pentamidine IV is given at a dose of 4 mg/kg daily. Adverse reactions are common and may be severe, including hypoglycemia, hypotension, cardiac arrhythmias and cumulative nephrotoxicity. Nausea, hyperkalemia, pancreatitis, hypokalemia, hypocalcemia, hyperglycemia and even permanent insulin-requiring diabetes mellitus have also been reported.<sup>116</sup> Because of the severity of some of its side effects, pentamidine IV should be given in the inpatient setting, over ≥1 hour, while the patient is supine and receiving IV hydration. Other nephrotoxic agents should be avoided. Close monitoring of renal function and serum glucose, calcium, and electrolyte concentrations should be performed at baseline and daily. Complete blood counts and liver function tests should also be obtained because of the risk of hepatitis and bone marrow suppression. More than 20% of patients given pentamidine experience recrudescence of PCP.<sup>11</sup>

Alternative regimens for mild-to-moderate PCP include TMP-dapsone, oral clindamycin-primaquine and atovaquone.

TMP-dapsone consists of oral TMP 5 mg/kg three times daily plus dapsone 100 mg daily. Side effects include gastrointestinal upset, rash, hemolytic anemia, and methemoglobinemia. TMP-dapsone is less likely to cause adverse reactions than TMP-SMX. Testing for G6PD deficiency should be obtained prior to initiating dapsone but therapy may be started before G6PD results become available.

Oral clindamycin-primaquine includes clindamycin 450 mg four times daily plus primaquine 15 mg per day. Side effects include rash, hemolytic anemia, neutropenia, methemoglobinemia, diarrhea, and *Clostridium difficile*-associated colitis. As with dapsone, G6PD testing should be performed prior to initiating therapy.

Atovaquone (suspension) is touted to have microbicidal activity towards *Pneumocystis* and is useful in mild-to-moderate PCP.<sup>11</sup> The drug is given as 750 mg twice daily and should be taken with food.<sup>117</sup>

## Addition of steroids

HIV-positive patients who have resting hypoxemia by oxygen saturation measurement and those with tachypnea should have arterial blood gas measurement. If partial pressure of oxygen (PO<sub>2</sub>) is ≤70 mmHg or the (A-a) DO<sub>2</sub> is ≥35 mmHg, prednisone should be given for 21 days as follows: 40 mg twice daily for

**Table 17-3** Treatment of Pneumocystosis

Regimen	Severity of disease			Adverse reactions
	Mild	Moderate	Severe	
<b>TMP-SMX♦</b> TMP: 15-20 mg/kg/day SMX: 75-100 mg/kg/day IV divided into 3-4 daily doses OR 2 DS tablets every 8 hours	✓	✓	✓	See Table 17-1. For neutropenia, use folinic acid (Leucovorin 5mg three times daily) and G-CSF until ANC > 1500/μL for 3 days. May add caspofungin standard dose in non-HIV positive patients.
Pentamidine IV 4mg/kg/day for 21 days	✓	✓	✓	Nephrotoxicity, hypotension, hyper/hypokalemia, hyper/hypoglycemia, hypocalcemia, pancreatitis, dysrhythmias, transaminitis, neutropenia. Start IV Pentamidine as inpatient; Avoid nephrotoxins; Monitor blood pressure, blood counts, renal and liver function tests, serum glucose, electrolytes, and calcium
Atovaquone 750 mg PO BID	✓	✓	—	See Table 17-1
Trimetrexate IV 45mg/m <sup>2</sup> /day plus leucovorin 20 mg/m <sup>2</sup> four times daily IV or PO	✓	✓	✓	Rash, neutropenia, thrombocytopenia, transaminase elevation. To prevent toxicity, continue leucovorin for 72 hours after Trimetrexate.
<b>TMP-Dapsone</b> TMP: 5 mg/kg PO TID Dapsone: 100 mg/day PO	✓	✓	—	Same as Table 17-1 for dapsone; Screen patients for G 6PD deficiency.
<b>Primaquine-Clindamycin</b> Primaquine: 15-30 mg/day <b>PO</b> Clindamycin: 600 mg every 8 hours IV <b>OR</b> 300-450 mg four times daily PO	✓	✓	✓	Rash, fever, diarrhea, methemoglobinemia, hemolytic anemia, neutropenia. Screen patients for G 6PD deficiency before starting primaquine

Key: PO: oral; G-CSF: granulocyte colony stimulating factor; ANC: absolute neutrophil count. TMP-SMX: trimethoprim-sulfamethoxazole; TMP: trimethoprim; DS: double strength; 160 mg of trimethoprim plus 800 mg of sulfamethoxazole;  
♦TMP-SMX effective against other infections including those caused by *Toxoplasma gondii*, *Isospora*, *Listeria*, *Nocardia*, *Salmonella*, *Legionella*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and many gram-negative bacilli.

5 days, followed by 40 mg daily for 5 days and then 20 mg daily for 11 days.<sup>8</sup> These patients should also be hospitalized because of the risk of clinical deterioration during the first 48–72 hours of therapy. Most non-HIV patients may have been receiving glucocorticoids at onset of their PCP. In such cases, the steroid dose should be maintained or even increased if PCP developed during a steroid taper. Steroids may also be considered if severe PCP develops in a patient who was not receiving steroids in the few weeks preceding the onset of PCP.

### Use of IV therapy

Patients with a seriously compromised pulmonary status (PO<sub>2</sub> <60 mmHg, (A-a) DO<sub>2</sub> >45 mmHg) or pending respiratory failure (PCO<sub>2</sub> normal or elevated in a patient with low PO<sub>2</sub> or high respiratory rate) should receive IV therapy.

IV TMP-SMX should be given at a dose of 15–20 mg/kg/day of the TMP component (TMP-SMX IV is supplied in a 1:5 ratio of TMP to SMX) divided in three or four doses.

**Suspect PCP if: fever, dyspnea, dry cough in a patient at risk PCP unlikely if any of the following is normal:**

- Oxygen saturation at rest and after exercise
- Serum LDH
- High resolution CT scan of the chest
- DLCO > 75% predicted
- Serum  $\beta$ -glucan\*



**If above abnormal and suggestive of PCP, confirm diagnosis with**

- Serum  $\beta$ -glucan
- BAL with Calcofluor white or GMS and if negative, use immunofluorescent antibody (in HIV patients, may start with IS / OPW / NPW sample); if negative, apply RT-PCR
- If intubated, may use endotracheal aspirates (as sensitive as a BAL sample)
- If BAL negative (low organism burden, aerosolized pentamidine, granulomatous PCP): obtain open lung biopsy; Avoid transthoracic needle biopsy (30% pneumothorax).



- Obtain ABGs to assess severity of disease, need for oral or IV therapy, and for admission
- Screen for G6PD deficiency if considering using dapsone or primaquine
- Stratify patients according to risk of respiratory failure and need for prednisone



Severity	Mild	Moderate	Severe
PO <sub>2</sub> mm Hg	> 70	≤ 70	< 60
A-a gradient, mm Hg	< 35	≥ 35	≥ 45
Respiratory failure	Absent ↓	Absent ↓	↑ Respiratory rate PCO <sub>2</sub> normal or ↑ despite hypoxia ↓
Drug	TMP-SMX	TMP-SMX	TMP-SMX*
Route	Oral	Oral	IV
Site of care	Outpatient	Inpatient	Inpatient
Prednisone	-	+	+
<b>Monitor O<sub>2</sub>, LDH and Serum <math>\beta</math>-glucan; if no response after 5–7 days</b>			
	Start IV ↓	Start IV ↓	Change to Pentamidine IV or Clindamycin/ primaquine ↓

IS: induced sputum; OPW: oropharyngeal washing; NPW: nasopharyngeal washing; RT-PCR: real time polymerase chain reaction; ABGs: Arterial blood gases;

A-a: alveolar-arterial oxygen gradient PO<sub>2</sub>: partial pressure of oxygen; PCO<sub>2</sub>:

\* Positive  $\beta$ -Glucan ≥ 80 pcg/ml; False +  $\beta$ -glucan: IVIG, Albumin IV, hemodialysis

\* In severe cases, may add caspofungin 75 mg loading dose then 50 mg IV daily

**Figure 17-4** PCP diagnosis and treatment.

Pentamidine IV (as above).

IV clindamycin-primaquine includes clindamycin 600 mg every 8 hours plus oral primaquine 30 mg daily.

## Treatment failure

Patients who fail to improve after 5–7 days of oral therapy should be switched to an IV regimen and those who experience treatment failure on IV therapy should receive an alternative IV regimen. Failure to respond or intolerance to either TMP-SMX or pentamidine should be treated with IV clindamycin and oral primaquine.

Because immunocompromised patients may be at risk for simultaneous infections, those experiencing treatment failure should undergo diagnostic testing to exclude concurrent pulmonary infections and, in certain settings, receive empiric therapy directed at the most likely infection while awaiting the results of diagnostic tests.

## Other agents

Antifungal agents have been demonstrated to be of little use in treatment of PCP.<sup>1</sup> Although the antifungal echinocandins, which inhibit  $\beta$ -1,3-glucan synthesis, have anti-*Pneumocystis* activity in animals,<sup>118</sup> they have little documented value in the treatment of human PCP and progression of *P. jiroveci* pneumonia has been reported in patients receiving echinocandin therapy.<sup>119</sup> One recent report described four cases of severe PCP in solid organ transplant recipients who received Caspofungin, an echinocandin, in combination with TMP-SMX with suggestion of clinical benefit.<sup>119a</sup> Whether the addition of agents targeting pneumocystis glucan synthetase (GSC1) thereby inhibiting fungal cell wall  $\beta$ -1,3-glucan synthesis, to conventional treatment will provide synergistic activity in severe PCP remains to be demonstrated.

Trimetrexate should be avoided if possible because of its toxicity. The PCP dose is 45 mg/m<sup>2</sup> daily with 20 mg/m<sup>2</sup> leucovorin (oral or IV) four times daily. To prevent toxicity, leucovorin should be continued for 72 hours after the course of trimetrexate has been completed. Adverse reactions of trimetrexate include neutropenia, thrombocytopenia, rash and elevated liver function tests.

## Special considerations

Characteristics of pneumocytosis in HIV-positive and non-HIV-positive patients

Significant differences exist between patients who are HIV positive and other populations at risk for PCP. These differences are shown in Table 17.4.

### Breakthrough PCP in patients on prophylaxis

Breakthrough PCP in patients who are compliant with TMP-SMX prophylaxis is very rare<sup>120</sup> and should be treated with pentamidine IV if the infection is severe and with clindamycin-primaquine or atovaquone if mild or moderate. Breakthrough PCP infections are more common with other prophylactic agents (dapsone, atovaquone or aerosolized pentamidine). In such cases, treatment with TMP-SMX is recommended while TMP-dapsone may also be used in mild-to-moderate PCP.<sup>121</sup>



**Table 17-4** Characteristics of Pneumocystosis in HIV positive and Non-HIV positive patients

	<b>HIV positive</b>	<b>Non-HIV positive</b>
Risk factors	CD4 < 200 cells/ $\mu$ l PCP prophylaxis not prescribed or adhered to HAART not prescribed or adhered to	CD4 < 200 cells/ $\mu$ l Systemic glucocorticoids for various disorders including inflammatory conditions, asthma, solid organ transplantation, hematopoietic stem cell transplantation, others. Inhaled glucocorticoids (asthmatic children) Cytotoxic chemotherapy Monoclonal antibodies: adalimumab, infliximab, etanercept, or rituximab for autoimmune diseases and cancer. Severe combined immunodeficiency syndrome (infants)
Onset of symptoms	Indolent	May be fulminant
Diagnosis	High burden of organisms: Induced sputum (IS) has good yield Perform BAL only if IS is negative	Low burden of organisms: - IS has low yield; use BAL as first diagnostic procedure. - Conventional stains (CW, GMS) may be negative; apply monoclonal antibody test. Granulomatous PCP may be particularly difficult to diagnose with BAL and may require open lung biopsy.
Response to therapy	Slow	More rapid (4–5 days)
Steroids	Indicated with moderate to severe PCP	Not indicated. Most non-HIV patients may have been receiving glucocorticoids. In such cases, maintain the steroid dose or increase it if PCP developed during steroid taper. Consider steroids if severe PCP develops in patient not on steroids
Duration of therapy	21 days because: - high burden of organisms, - slower response time, - early relapses after 14 day treatment.	14 days because: - low burden of organisms - rapid response time - few relapses after 14 day treatment.
Outcome	Good	Not as good as in HIV positive patients
Key: PCP: Pneumocystis pneumonia; IS: induced sputum; BAL: bronchoalveolar lavage; Conventional stains (CW: calcofluor white; GMS: gomori methenamine silver)		

### Pregnancy

Sulfa drugs and dapsone given near delivery may increase the risk of kernicterus while pentamidine, though not teratogenic, may cause embryonic toxicity and death.<sup>122</sup> Trimetrexate should be avoided in pregnancy<sup>121</sup> and pregnant women receiving prednisone during the third trimester should be closely monitored for gestational diabetes.<sup>121</sup>

### HAART therapy in HIV-infected patients

Patients receiving HAART should continue their regimen. On the other hand, treatment-naïve patients should not start HAART until after PCP therapy has been completed. Earlier

start of HAART may result in clinical deterioration as a result of an immune reconstitution and inflammation syndrome.

### Secondary PCP prophylaxis

Prophylaxis should be started following completion of PCP therapy.

### Resistance

Recent reports have suggested the emergence of *Pneumocystis* resistant to sulfonamides.<sup>72,73</sup> Mutations found in the *Pneumocystis* DHPS gene, which are homologous to mutations causing sulfa resistance in other pathogens (e.g., *Plasmodium*

*falciiparum*, *Streptococcus pneumoniae*, *Neisseria meningitidis*), are associated with previous exposure to sulfa drugs and with an increased risk of death from PCP.<sup>3,9,11,72,73,123</sup> The presence of multiple mutations in a gene locus that otherwise lacks variability is indicative of a pronounced selective pressure.<sup>72,73</sup> Recently, mutations in the cytochrome b gene have been identified in the mitochondria of *Pneumocystis* and shown to confer resistance to atovaquone.<sup>124</sup> Although such a degree of selection is not itself an indication of resistance, it does suggest that resistance may be emerging. Potential drug-resistant strains of *Pneumocystis* could present more of a problem for HIV-negative immunosuppressed patients than HIV patients who can recover immune function thanks to HAART.<sup>11,72,73</sup> The development of new anti-*Pneumocystis* strategies remains a priority.

## Summary

PCP remains a serious cause of morbidity and mortality in both HIV-infected and other immunocompromised individuals. Although HAART has produced an impressive decrease in the incidence of PCP, this decline, evident only in the industrialized world,<sup>6</sup> may be short-lived. The growing emergence and transmission of antiretroviral-resistant strains of HIV are such that if new antiviral agents do not become available, the number of patients with resistant virus and opportunistic infections, including PCP, will continue to climb.

The application of PCR-based detection and strain typing methods has provided new insights into the epidemiology of PCP, including the carriage and transmission of organisms between susceptible hosts as well as probable acquisition from the environment. It is now understood that lung injury and respiratory compromise during PCP are mediated by marked inflammatory responses to the organisms and that optimal management of the disease requires administration of corticosteroids to suppress lung inflammation as well as the use of TMX to inhibit the growth of the organism.

The broad use of sulfa agents for prophylaxis and treatment of PCP has placed extraordinary selective pressure on the organism, with a resultant induction of mutations in the *P. jirovecii* DHPS gene. Although the presence of DHPS mutations has been associated with failure of prophylaxis, presently such mutant strains appear to be treatable with the higher doses of TMX used in therapy.

Ongoing studies of this opportunistic fungal pathogen should continue to provide clinically useful insights that will facilitate efforts to prevent and treat this potentially fatal infection in the immunocompromised patient population.

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# Anomalous fungal and fungal-like infections: lacaziosis, pythiosis, and rhinosporidiosis

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## Lacaziosis

### Etiology

The etiologic agent of Jorge Lobo's disease (lacaziosis), *Lacazia loboi* is an uncultivated fungal pathogen of humans and dolphins causing cutaneous and subcutaneous infections and, rarely, visceral involvement. This anomalous pathogen is restricted to Mexico, Central America, and South America. However, three imported cases diagnosed in Europe and North America have been recorded.<sup>1-3</sup> Cases in dolphins in the coasts of France<sup>4</sup> and the USA<sup>5</sup> with transmission to aquarium personnel have also been documented. The first case was diagnosed in 1930 by Jorge Lobo<sup>6</sup> in a Brazilian human patient with cutaneous parakeleoidal lesions. Since then, hundreds of new cases have been recorded in Latin America, but Brazil has the highest incidence.<sup>7,8</sup>

The etiologic and epidemiologic features of *L. loboi* have always been surrounded by controversies. Since this anomalous pathogen resists culture, these controversies were only recently resolved with the use of molecular methodologies.<sup>9-11</sup>

*Lacazia loboi* has been known by various names such as: *Blastomyces brasiliensis*, *B. loboi*, *Glenosporella loboi*, *Glenosporopsis amazonica*, *Loboa loboi*, *Lobomyces loboi*, and *Paracoccidioides loboi*. In addition, the disease has also been known by controversial epithets such as lobomycosis, a name derived from the genus *Lobomyces*.<sup>12</sup> Another popular name for the infections caused by *L. loboi* has been "Lobo's disease." This is a short version of the original "Jorge Lobo's disease" terminology. Some investigators claimed to have cultured the etiologic agent of this unusual disease, which added more confusion about *L. loboi* in vitro identity. However, those isolates were later found to be common contaminants or strains of *P. brasiliensis*.<sup>7,8</sup> The term "lacaziosis" was recently introduced by Vilela et al.<sup>11</sup> They argue that in medical mycology the use of the genus to name a disease is common practice. Since the genus *Lobomyces* is now a synonym of the genus *Lacazia*, the term "lobomycosis" should be considered an obsolete synonym of lacaziosis and Jorge Lobo's disease.

Herr et al<sup>9</sup> reported that *L. loboi* is phylogenitically linked to *P. brasiliensis* and to the other dimorphic fungal Onygenales. This pioneering study suggests that *L. loboi* may be a dimorphic

fungus with a mycelial form in nature. The placement of *L. loboi* within the dimorphic Onygenales was later confirmed by Vilela et al<sup>11</sup> using the DNA sequences coding the gp43-like protein of *L. loboi*. These studies ended 70 years of taxonomic ambiguities and validated the genus *Lacazia*, originally proposed by Taborda et al in 1999.<sup>13</sup> The development of a molecular model to study *L. loboi*<sup>11,14</sup> suggests that other unusual features of this restricted microbe could soon be unveiled.

### Distribution and epidemiology

The infections caused by *L. loboi* are restricted to Latin America.<sup>7,8,15</sup> Some reports of the disease in other geographic areas were found later to be related to fungal etiologies other than *L. loboi*.<sup>8,16</sup> In Latin America only Chile, El Salvador, Guatemala, and Nicaragua have yet to report the disease. According to Lacaz et al,<sup>7</sup> around 500 cases of lacaziosis have been documented since 1931. Interestingly, 60% of these have been diagnosed in or near the Amazon forest of Brazil. Countries such as Colombia, French Guiana, Surinam, and Venezuela, which share the Amazon borders with Brazil, have together diagnosed 24.6% of the cases, second only to Brazil. Imported cases of the disease outside Latin America have also been diagnosed in Canada,<sup>2</sup> France,<sup>3</sup> and the USA.<sup>1</sup> However, the patients had visited the endemic areas of the disease. In addition, anomalous cases of putative transmission of *L. loboi* from an infected dolphin to aquarium personnel were recorded in France and in Hawaii, USA.<sup>4,5</sup>

The disease occurs in areas with climates resembling the Amazon River basin and rainforest regions. In addition, lacaziosis has been diagnosed in dolphins.<sup>5,17,18</sup> Since the disease is restricted to Latin America, it is believed that dolphins may be infected while swimming near the estuaries of large South American rivers. This fact was used by Ajello<sup>19</sup> to propose a new category of fungal pathogen: the hydrophilic pathogenic fungi. His proposal predicted that *L. loboi* might be a hydrophilic fungus; thus humans and dolphins could be infected after traumatic implantation of the pathogen from water or wet environments containing the propagules of this fungus. However, based on the phylogenetic placement of *L. loboi* in the Onygenales, it has also been postulated that *L. loboi* might develop conidia similar to those observed in



*Blastomyces dermatitidis* and *P. brasiliensis*. Thus, following skin trauma these forms may enter the host tissues from contaminated soil, plant detritus, and organic material.

## Pathogenesis and clinical features

Little is known about the pathogenesis of this non-life threatening disease. Based on experimental human inoculation<sup>12</sup> and mouse model experiments,<sup>20-22</sup> it is believed that the disease may be acquired after skin trauma and exposure to *L. loboi* propagules. Environmental (geography, large forest and rivers) and host (age, sex, ethnicity, occupation, immune status) factors might also play key roles in acquiring infection. It is well known that lacaziosis occurs in apparently healthy hosts. A genetic predisposition theory regarding a Brazilian Indian tribe heavily affected by this disease was soon abandoned after the tribe was moved to a different geographic location and the number of cases dramatically decreased.<sup>7</sup> Based on numerous reports of the disease after skin trauma with plant parts and/or insect bites, it has been suggested that *L. loboi* gains entrance to the tissues after skin damage and exposure to organic materials such as soil, plants, and/or water contaminated with *L. loboi*.<sup>19,23,24</sup>

Once in the infected tissues, *L. loboi* develops very slowly and could take months or years to enlarge the original wound to the typical parakeloidal lesion (keloidiform blastomycosis). After successful implantation, lesions may develop on the patient's arms, back, ears, face, legs, and shoulders, but the most frequent clinical location is the ears, followed by the face and the extremities (Fig. 18-1A). The disease is more commonly diagnosed in males than in females with an age range of 20–40 years. Initial signs of infection include mild pruritus at the inoculation site slowly evolving to warty lesions.<sup>7,12,23,25,26</sup> Visceral involvement of *L. loboi* has not yet been described. However, rare cases of dissemination from cutaneous lesions to lymphatic tissues and reproductive organs have been recorded.<sup>7,8,24</sup> Autoinoculation evolving to multicentric lesions has also been recorded in some patients.<sup>8,23,27,28</sup>

While lacaziosis is more frequent in indigenous people of the endemic areas, Rodriguez-Toro and Tellez<sup>29</sup> and Wiersema and Niewel<sup>30</sup> describe the infection in black males from Colombia and Surinam. In addition, visitors traveling to the endemic areas of Latin American could also acquire the infection, as was recently reported.<sup>1-3</sup>

Silva<sup>28</sup> recognized at least five clinical manifestations of the disease, including the typical parakeloidal lesion, and four



**Figure 18-1** The figure depicts three different Brazilian patients with lacaziosis. (A) Infiltrative nodular lesion of 5 years' duration on the patient's right ear. Ears are the most common anatomic location of the disease. (B,C) Two patients with exacerbat multicentric parakeloidal lesions. Abdominal lacaziosis such as that in panel B is unusual. (Courtesy of Drs P. Rosa and S. Talhari.)

other forms: infiltrative, gummatous, ulcerated and verrucous. Machado,<sup>26</sup> on the other hand, described two more comprehensive clinical manifestations of lacaziosis: (1) a hyperergic stage, which includes the macular, gummatous, and nodular forms, and (2) a hypoergic stage, which includes the parakeloidal and verrucous forms (Figs 18-1B, C). The differential diagnosis comprises skin diseases such as chromoblastomycosis, leishmaniasis, leprosy, various neoplasias, paracoccidioidomycosis, and other similar skin conditions.<sup>8,23,25</sup>

## Pathology, laboratory diagnosis and treatment

In histopathologic sections of the infected tissues, a diffuse histiocytic reaction with large numbers of giant cells, fibrosis, and a granulomatous reaction with the formation of pseudotubercles have been described (Fig. 18-2A).<sup>7,24,31,32</sup> This reaction could damage the subcutaneous connective tissues including local nerves, which could explain the anesthetic effect of some large lesions.<sup>25,27,29</sup> Within the inflammatory response the presence of numerous unicellular yeast-like cells of *L. loboi* in chains, some of them in the cytoplasm of giant cells and macrophages, is the main feature of the infection (Fig. 18-2A).<sup>24,31</sup> The yeast-like cells are spheroid, 6.0–13.0 × 5.0–11.0 µm in diameter and uniform in shape, which is very similar to the parasitic stage of *P. brasiliensis*.<sup>7</sup> Apparently, when the yeast-like cells of *L. loboi* divide they remain attached to the previous cell by the formation of a small tube, thus forming long chains of 3–15 or more cells, a feature more commonly observed in silver-stained tissue sections (Fig. 18-2B). About 20–46% of the yeast-like cells were found to be dead in the infected tissues, a finding which could be linked to the chronicity of the disease since it appears that the dead cells of this pathogen are more difficult to remove by the cellular response.<sup>22,27,33</sup>

Samples collected for mycologic diagnosis should be cultured on different media to aid in the differential diagnosis with other etiologies. However, this practice is uncommon since most laboratory workers believe that *L. loboi* is non-culturable, an approach that may lead to serious difficulties regarding the differential diagnosis. Wet mount preparations in 10% KOH containing mostly single yeast-like cells 3.0–12 µm in diameter, with a few of them in chain of 2–3 cells (Fig. 18-2C). Several dancing protoplasmic granules can also be observed within some of the yeast-like cells. Clinical samples of cases suspected of lacaziosis can also be processed by staining smears of the tissues with Giemsa, Wright, and/or Gram stains. Serologic test using antigens of *P. brasiliensis* consistently detected cross-reacting antibodies in patients with lacaziosis.<sup>14,34</sup> Successful experimental infection in mice and other lower animals, and also in humans, has been reported.<sup>7</sup> Several generations of mice have been successfully maintained after experimental infection with samples from humans and dolphins diagnosed with the disease.<sup>20,24</sup> Although Haubold et al.<sup>35</sup> reported size differences of the yeast-like cells between isolates from human and dolphin tissues infected with *L. loboi*, the clinical similarities of mice inoculated with the pathogen from both host species have prompted speculation about a possible common etiology in humans and dolphins.

The management of lacaziosis is difficult because patients respond poorly to most antifungal drugs.<sup>8,23,30,36</sup> Curiously, spontaneous resolutions in Brazilian patients with the disease

have been reported.<sup>26</sup> The use of clofazimine (100–200 mg/day) has been more successful than antifungal drugs such as amphotericin B, ketoconazole (400 mg/day), 5-fluorocytosine (100–200 mg/kg) and other drugs.<sup>8,37</sup> Surgery and electrodesiccation have practical applications, mostly in the early stages of the disease.<sup>8</sup> However, infected populations are often poor and without access to adequate healthcare. Thus, most cases evolve to large multicentric lesions difficult to remove by surgical intervention. In addition, surgery is very invasive and has a high recurrence rate.

## Pythiosis

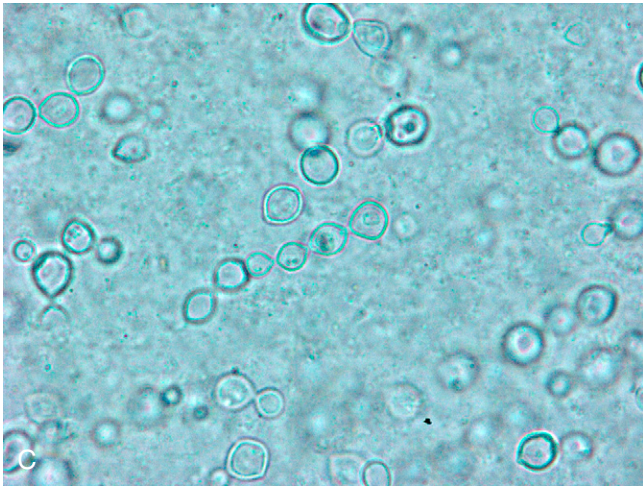
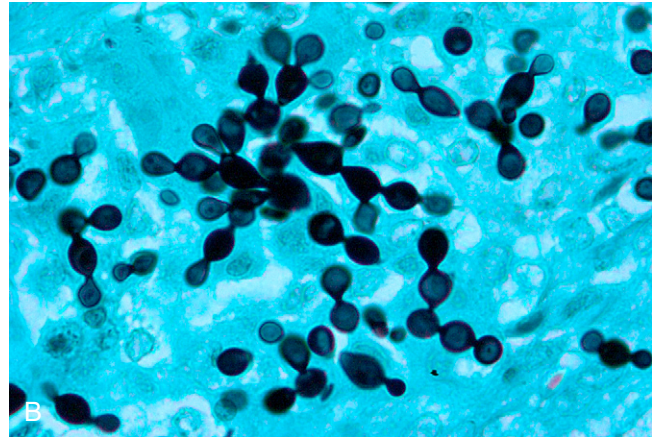
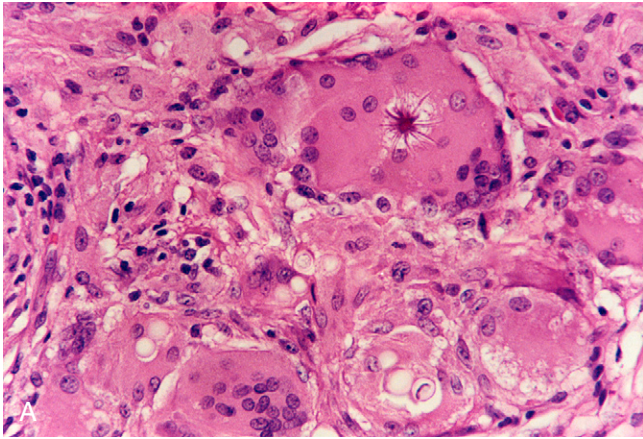
### Etiology

*Pythium insidiosum* is the etiologic agent of pythiosis, an arterial, cutaneous, subcutaneous, intestinal and, more rarely, systemic infection of mammals in the tropical and subtropical areas of the world.<sup>38</sup> *Pythium* species are well-known hydrophilic plant pathogens inflicting serious damage on economically important plant crops.<sup>39</sup> Taxonomically, *Pythium* spp. are classified in the protist kingdom Stramenopila along with plant and fish pathogens and saprotrophic microbes such as *Achlya*, *Lagenidium*, *Phytophthora*, and *Saprolegnia* species, and other important zoosporic microbes.<sup>39</sup> The development of sparsely septate hyphae and the formation of biflagellated zoconidia were used to place the Peronosporomycetes (Oomycetes) within the fungi. However, recent phylogenetic studies have moved them into the Stramenopila near the green algae and some lower plants.<sup>38,39</sup>

The genus *Pythium* comprises more than 200 species but only *P. insidiosum* has been reported to cause disease in mammals.<sup>38,40,41</sup> A *Lagenidium* sp. recently recovered from some dogs with pythiosis-like lesions is still awaiting verification.<sup>42</sup> However, it is thought that these canine isolates may not belong to the genus *Lagenidium*, but are more related to the cryptic phylogenetic strains of *P. insidiosum*. In addition, an anomalous isolate of *P. insidiosum* was recovered from an infected mosquito in India.<sup>40,41</sup> This unexpected finding suggests that, in addition to plants and mammals, *P. insidiosum* may also affect mosquitoes, a feature commonly seen with other Peronosporomycetes that parasitize insects.<sup>39</sup>

The first cases of equine pythiosis were recorded during the 1890s in India, Indonesia, other eastern Pacific islands and the USA (Florida).<sup>38</sup> In the USA the infection was considered an exotic disease of equines until several investigators described numerous cases in Texas and called attention to this pathology. Similar reports were published during the 1900s in Australia, Japan, New Guinea, New Zealand, and Latin America.<sup>38</sup> Until 1971 the disease was only known to occur in horses. However, several cases in dogs were diagnosed in the 1970s and 1980s.<sup>43</sup> The first human case was recorded in Thailand in 1986.<sup>44</sup> Soon after, human cases were diagnosed in Australia, Brazil, Haiti, New Zealand, and USA. Thailand has the most recorded cases, with some 100 patients with the disease,<sup>45</sup> followed by the USA with 11 reported cases. Two of the 11 cases have already been published;<sup>46,47</sup> four unpublished cases include a man with pythiosis keratitis from Philadelphia,<sup>40,41</sup> at least two subcutaneous cases in adults and one orbital case in a Tennessee girl (data not yet published), and





**Figure 18-2** (A) Hematoxylin and eosin-stained histologic section from a case of lacaziosis. Fibrosis and various giant cells, containing numerous poorly stained yeast-like cells uniform in size, are the main histopathologic features of the disease. The largest giant cell on the upper part of the panel contains an asteroid body sometimes found in histopathologic preparations from cases of lacaziosis (courtesy of Dr P. Rosa.) (B) Gomori methenamine silver-stained section with numerous yeast-like cells connected by slender tubes forming chains of five or more cells (40 $\times$ ). (C) Chain of yeast-like cells from a case of lacaziosis in 10% KOH. Small vesicles can be observed in the cytoplasm of some cells (40 $\times$ ).

five orbital cases in children misdiagnosed as fungal infections, recently reclassified as putative cases of pythiosis.<sup>48</sup>

*Pythium* spp. colonize a wide variety of environments, using plants or plant detritus to complete their life cycle in nature.<sup>49</sup> Various studies have suggested that *P. insidiosum* follows this model in the endemic areas of pythiosis.<sup>38,50</sup> According to recent studies, plants such as gramineas and lilies in wet areas could be colonized by *P. insidiosum* in nature, developing biflagellated zooconidia. It is not known if this mammalian pathogen in any way harms the plant. The zooconidia swim to find new host plants and then encyst, develop hyphae and, if conditions are ideal, develop sporangia with zooconidia and the life cycle is reinitiated (see Fig. 18-4D). However, reports of the disease in animals and humans not exposed to the wet land fomites indicate that *P. insidiosum* may develop resistant conidia, and also that hosts may acquire the infection via propagules other than zooconidia.<sup>49</sup>

## Distribution and epidemiology

Cases of pythiosis in humans and lower animals are usually reported in the tropical and subtropical areas of the world.<sup>38</sup> However, cases in the temperate areas of Japan, South Korea, and the USA (Illinois, New York, Wisconsin) have also been documented. Pythiosis is more commonly diagnosed in Australia,

Asia and Latin America. The tropical location of Africa seems an ideal environment for pythiosis. However, only one case of dog pythiosis from Mali has so far been published.<sup>51</sup> Apart from a case of equine pythiosis recorded in 1867 in France, Europe has yet to report the infection.<sup>38</sup> In the USA the disease is more prevalent in the coastal states surrounding the Gulf of Mexico, but the disease has also been diagnosed in Arizona, California, New Mexico and as far north as Illinois, New Jersey, New York, Virginia, and Wisconsin. In Latin America the disease is known to occur from Mexico to Argentina. The freshwater areas of Brazil “Pantanal” are believed to be the most favorable environments for *P. insidiosum*. In Australia, New Guinea, and New Zealand the disease is more prevalent in the coastal areas, whereas in Thailand pythiosis is more commonly reported inland.<sup>44,45,52</sup> In Japan and South Korea the disease occurs in the southern areas only in horses and dogs.<sup>53</sup>

Ravishanker et al<sup>54</sup> showed that *P. insidiosum*’s hyphae do not exert enough force to penetrate healthy skin. Thus, a traumatic implantation of *P. insidiosum* propagules is required for infection. Several investigators have demonstrated that *P. insidiosum*’s zooconidia have an unusual tropism for both mammalian flesh and plant foliage.<sup>49,50</sup> This behavior was used by some to incriminate the zooconidia of *P. insidiosum* as the infecting units. These investigators reported that encysted zooconidia expressed a sticky material that allows the



pathogen to remain attached to its host. The attached encysted zooconidia then develop germ tubes which, stimulated by the host temperature, actively penetrate the infected tissues. However, diagnosed cases of pythiosis in the absence of wet environments have led to speculation that other propagules of *P. insidiosum*, including resistant conidia and hyphal elements, may also play the infecting unit role.<sup>49</sup> Thus, susceptible individuals inhabiting endemic areas could acquire the infection after a traumatic event, exposure to the propagules of *P. insidiosum* and development of penetrating hyphae.<sup>54</sup>

## Pathogenesis and clinical features

It is believed that most individuals in endemic areas have contacted the propagules of *P. insidiosum* yet remain asymptomatic. Serologic tests from these populations showed low anti-*P. insidiosum* antibody titers, which seem to support this view.<sup>53,55</sup> Pythiosis is usually diagnosed in apparently healthy individuals.<sup>44,46,47,56</sup> Oddly, in Thai patients the disease occurs more frequently in individuals with thalassemia and similar blood disorders,<sup>52,57,58</sup> which is a characteristic absent in humans and lower animals with the disease in other geographic locations.<sup>47,56</sup> The fact that most Thai patients have arterial pythiosis, an unusual form of the disease rarely reported in other areas, has been used to explain this.<sup>45</sup> In spite of this, it appears that most humans and lower animals inhabiting endemic areas seem resistant to the infection. Beside humans, the disease has also been reported in bovines, canines, equines, felines, and ovines, and in several captive animals.<sup>42</sup> Cases of animal-human-human-animal transmission have yet to be reported. Colloquial reports of dogs eating flesh from infected horses without developing the infection exist. These observations suggest that *P. insidiosum* may only be acquired from natural environments contaminated with the pathogen. Experimental pythiosis has not yet been possible in naturally infected hosts, but has been induced in rabbits.<sup>59</sup>

*Pythium insidiosum* upon tissue invasion triggers an inflammatory response composed mostly of eosinophils, mast cells, giant cells and other inflammatory components.<sup>44</sup> It has been postulated that the penetrating hyphae express exoantigens that lock the immune system into a T helper 2 (Th2) response.<sup>53</sup> These exoantigens are believed to trigger the expression of IL-4, IL-5 and large quantities of IgE, which in turn activate the release of numerous eosinophils and mast cells, both incriminated in the tissue damage observed in infected areas.<sup>53</sup> The tropism toward the hyphae by the released eosinophils and mast cells is apparently an evolutionary strategy of *P. insidiosum* to conceal important antigens from the immune system.<sup>53</sup> The released eosinophils degranulate around the hyphal elements of *P. insidiosum*, forming the Splendore-Hoeppli phenomenon.<sup>44,53</sup> In horses this phenomenon is so intense that the formation of large stony masses, termed “kunkers,” is the main feature of equine pythiosis. However, these hard masses are absent in other animal species with the disease, including humans.<sup>44,45,60</sup>

The clinical signs of pythiosis observed in humans and lower animals are studied according to their anatomic location. For example, superficial infections cause keratitis involving the cornea,<sup>44,61-63</sup> cutaneous and subcutaneous pythiosis include orbital pythiosis and bone involvement,<sup>46,47,56,60</sup> arterial pythiosis affects large arteries of the lower limbs,<sup>45,52,57</sup> intestinal pythiosis occurs mostly in canines and some equines,<sup>64,65</sup>

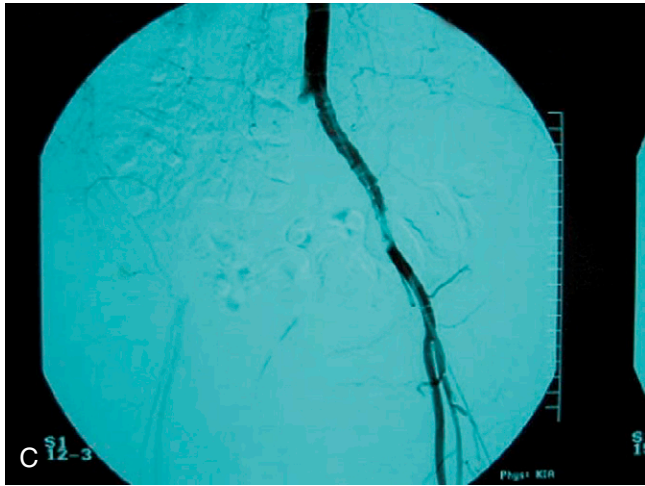
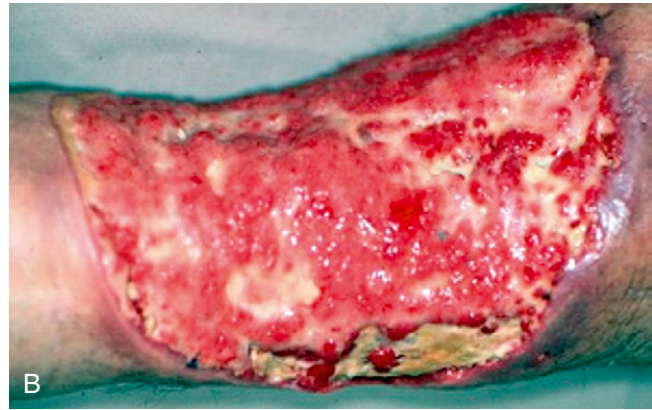
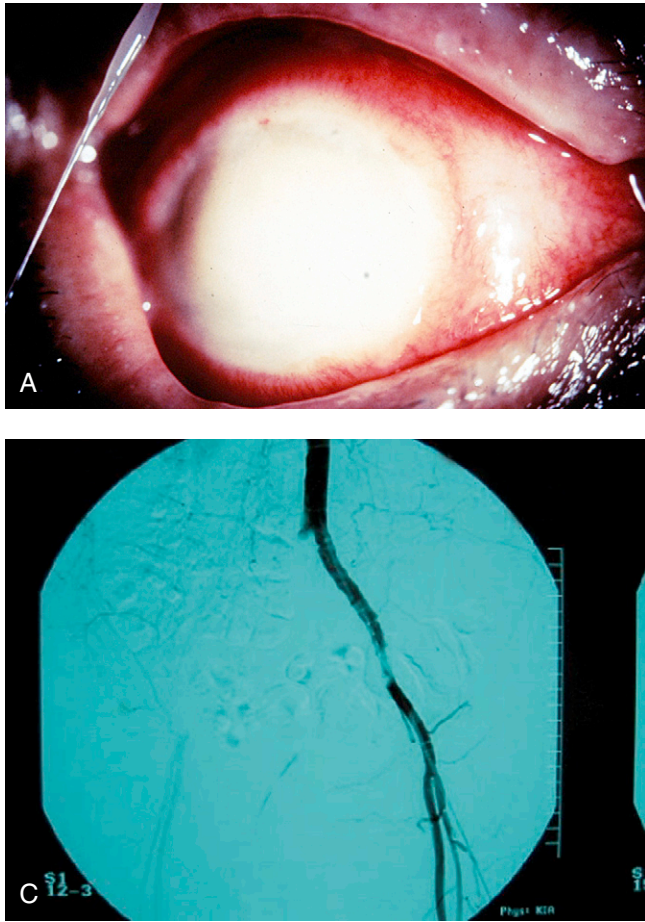
and more rarely, disseminated pythiosis involves the internal organs in humans with terminal arterial pythiosis.<sup>44,45</sup> Keratitis caused by *P. insidiosum* is apparently more common than initially suspected and it displays the same clinical features as that caused by the filamentous fungi.<sup>45</sup> Thus, the differential diagnosis is challenging since few laboratories are familiar with this emerging pathogen. Keratitis is more frequent in adults and the symptoms usually begin after trauma to the cornea with plant detritus that contains *P. insidiosum*. Subsequently, painful photophobia, tearing, and hypopyon, followed by the formation of corneal ulcers and endophthalmitis, are the main features of the infection (Fig. 18-3A). This form of pythiosis is particularly refractory to antifungal management and the patient is usually treated by surgical enucleation of the infected eye.<sup>44,45</sup>

Cutaneous and subcutaneous pythiosis have been reported in humans and lower animals after contact with stagnant water or other wetland fomites in endemic areas. Due to the morphologic features of *P. insidiosum* hyphae, this clinical form is frequently misdiagnosed as zygomycosis or as other fungal infections caused by the filamentous fungi, for example orbital aspergillosis (Table 18-1). The initial cutaneous clinical signs are small itchy papules that rapidly progress to form large painful lesions that may later ulcerate or disseminate to the adjacent subcutaneous tissues or bones, or both (Fig. 18-3B).<sup>46,47,60</sup> Orbital pythiosis is more commonly diagnosed in apparently healthy children in Australia and the USA.<sup>46,47,56</sup> The initial infection involves the formation of small itchy papules that later develop into periorbital cellulitis.<sup>56</sup> If left untreated, *P. insidiosum* may invade subcutaneous tissues, including nearby arteries, which is life threatening.<sup>45,48,52</sup> The differential diagnosis involves laboratory discrimination with the fungi, particularly the etiologic agents of zygomycosis, aspergillosis and other filamentous fungi.

Arterial and disseminated pythiosis is more frequently diagnosed in Thailand.<sup>44,45,57</sup> The infection begins with small skin papules at the inoculation site.<sup>44</sup> It is not well understood why *P. insidiosum*'s hyphae in some patients consistently remain in the subcutaneous tissues, whereas in others they propagate to the nearby arteries, causing vasculitis. Arterial invasion is followed by endothelial necrosis, which later develops into ischemia, swelling, muscular dystrophy, skin discoloration, and eventually gangrene of the infected limbs.<sup>44,45,52</sup> If untreated, patients may develop thrombosis of the large arteries, resulting in progressive ascending arteritis with the formation of large aneurysms (Fig. 18-3C).<sup>45</sup> Progressive ascending pythiosis could also involve other large vessels such as the iliac and renal arteries, and the abdominal aorta. Once the hyphae reach the abdominal aorta, the pathogen may disseminate to the internal organs, usually a fatal clinical event.<sup>44,45</sup> Arterial pythiosis should be differentiated from arteriosclerosis, diabetes mellitus and other vascular diseases.

## Pathology, laboratory diagnosis and treatment

The most prominent findings in histopathologic preparations stained with hematoxylin and eosin (H&E) from cases of keratitis are corneal and choroidal chronic inflammation with a cellular infiltrate composed of neutrophils, plasma cells, lymphocytes, macrophages, eosinophils and, rarely, giant cells.<sup>44,47</sup> Hyphal elements of *P. insidiosum* are very difficult



**Figure 18-3** The three major clinical manifestations of infections caused by *Pythium insidiosum*. (A) An advanced case of keratitis in a Thai patient with pythiosis. Typical ulcerated hyphate infiltrate within the cornea is evident. (B) A Brazilian patient with subcutaneous pythiosis. This patient shows extensive ulcerated tissue with necrosis that responded poorly to antifungal therapy. In Thailand vascular pythiosis is common. (C) Angiography of a Thai patient with advanced vascular pythiosis showing occlusion of the upper right iliac artery very close to the aorta. (Courtesy of Drs E. Bagagli, T. Krajaejun, and S. Kunavisarut.)

to detect in H&E preparations.<sup>44</sup> However, hyphae stained with Gomori methenamine silver appear as sparsely septate elements of 3–10  $\mu$ m in diameter with occasional right-angled branches.<sup>7,44,47</sup>

H&E-stained tissue sections from cases of cutaneous and subcutaneous pythiosis, including patients with the orbital form, show mostly epidermal acanthosis with a granulomatous infiltrate composed of neutrophils, plasma cells, lymphocytes, giant cells and eosinophils (Fig. 18-4A).<sup>44</sup> Although the eosinophilic reaction is less evident than in cases of equine pythiosis and human entomophthoromycosis (see Table 18-1), the eosinophils in the subcutaneous tissues tend to degranulate around *P. insidiosum*'s hyphae, forming the Splendore–Hoepli phenomenon. In ulcerated tissue, microabscesses, necrosis and a mixture of chronic and acute inflammatory infiltrate are commonly observed.<sup>47</sup> Hyphal elements of *P. insidiosum* surrounded by the Splendore–Hoepli phenomenon can be found at the center of these microabscesses.<sup>44,45</sup> In silver-stained preparations short transversal and longitudinal hyphal elements of *P. insidiosum* may be found (Fig. 18-4B).

Systemic and vascular pythiosis is associated with main arterial invasion of the lower limbs.<sup>44,45</sup> The infected vessels show a dramatic acute inflammatory response consisting of neutrophils, lymphocytes, plasma cells and necrosis of the tunica, as well as the outer and inner components of the arterial wall.<sup>45</sup> In addition, thrombosis in the lumen of the infected

arteries is a common feature. The formation of large aneurysms of the main arteries, including the aorta, is usually observed in the last stages of the disease (see Fig. 18-C).<sup>44,52</sup> In H&E-stained sections, the hyphal elements of *P. insidiosum* are difficult to detect within the infected arteries. However, silver stain methods reveal large quantities of short, sparsely septate, branching hyphal elements of *P. insidiosum* in the arterial cell wall and around the thrombus. In disseminated cases involving internal organs, the hyphal elements of *P. insidiosum* are only detected with Gomori methenamine silver.<sup>44</sup> This feature of *P. insidiosum* hyphae is in sharp contrast to that in the etiologic agents of zygomycosis, where hyphal elements are easily observed in H&E-stained tissues (see Table 18-1).<sup>8</sup> In addition, specific in situ histochemical and immunologic tests for the diagnosis of pythiosis have been successfully used in the past 10 years.<sup>43,55,64,66</sup>

Despite the successful development of diagnostic tools for pythiosis, most clinical laboratories are unfamiliar with the isolation and identification of *P. insidiosum*. Thus, cultures of *P. insidiosum* have usually been misidentified as fungal pathogens.<sup>8</sup> Scrapings or swab samples from cases of keratitis are submitted to the laboratory. Usually, the clinical material would be examined microscopically in 10% KOH and cultured on 2% Sabouraud dextrose agar followed by incubation at 37°C and at room temperature to rule out a fungal etiology. Unfortunately, the hyaline, sparsely septate hyphal elements

**Table 18-1** Similarities and differences shared by *Pythium insidiosum* and the etiologic agents Of zygomycosis, entomophthoromycosis and aspergillosis affecting humans (modified from Mendoza et al<sup>48</sup>)

	<b>Zygomycosis</b>	<b>Entomophthoromycosis</b>	<b>Aspergillosis</b>	<b>Pythiosis</b>
Host	Compromised	Apparently normal hosts	Compromised. Few cases in healthy adults around the face	Apparently healthy hosts. Vascular thalassemic Thai patients
Duration of illness	Rapidly progressive, usually fatal	Slow and progressive, remains indolent for years	Rapidly progressive, could be fatal	Protracted, 5–9 months, usually fatal
Inflammatory response	Suppurative marked necrosis, neutrophils, acute	Granulomatous, marked eosinophilia, Splendore–Hoeppli, chronic	Necrotizing granulomas of the face, acute but could become chronic	Granulomatous, marked eosinophilia, Splendore–Hoeppli, acute–chronic
Arterial invasion	Very common	Unusual	Unusual	Common
Management	Antifungal drugs	Antifungal drugs, iodides	Antifungal drugs	Poor response to antifungal drugs, immunotherapy, iodides, surgery
Features of the hyphae	10–15 µm D, H&E stained aseptate ribbon type	7–20 µm D, H&E stained aseptate ribbon type	3–6 µm D, H&E stained septate, uniform size	2.5–10 µm D, do not stain in H&E, sparsely septate, uniform in size
Epidemiology	Ubiquitous	Tropical and subtropical	Ubiquitous	Tropical, subtropical, and temperate areas
Etiology	Species of <i>Absidia</i> , <i>Mortierella</i> , <i>Mucor</i> , <i>Rhizopus</i> , <i>Saksenaia</i> , others	<i>Conidiobolus coronatus</i> <i>Basidiobolus haptosporus</i>	<i>Aspergillus fumigatus</i> , <i>A. flavus</i> , <i>A. niger</i> , others	<i>Pythium insidiosum</i>
Taxonomy	Kingdom Fungi	Kingdom Fungi	Kingdom Fungi	Kingdom Stramenopila, Protist

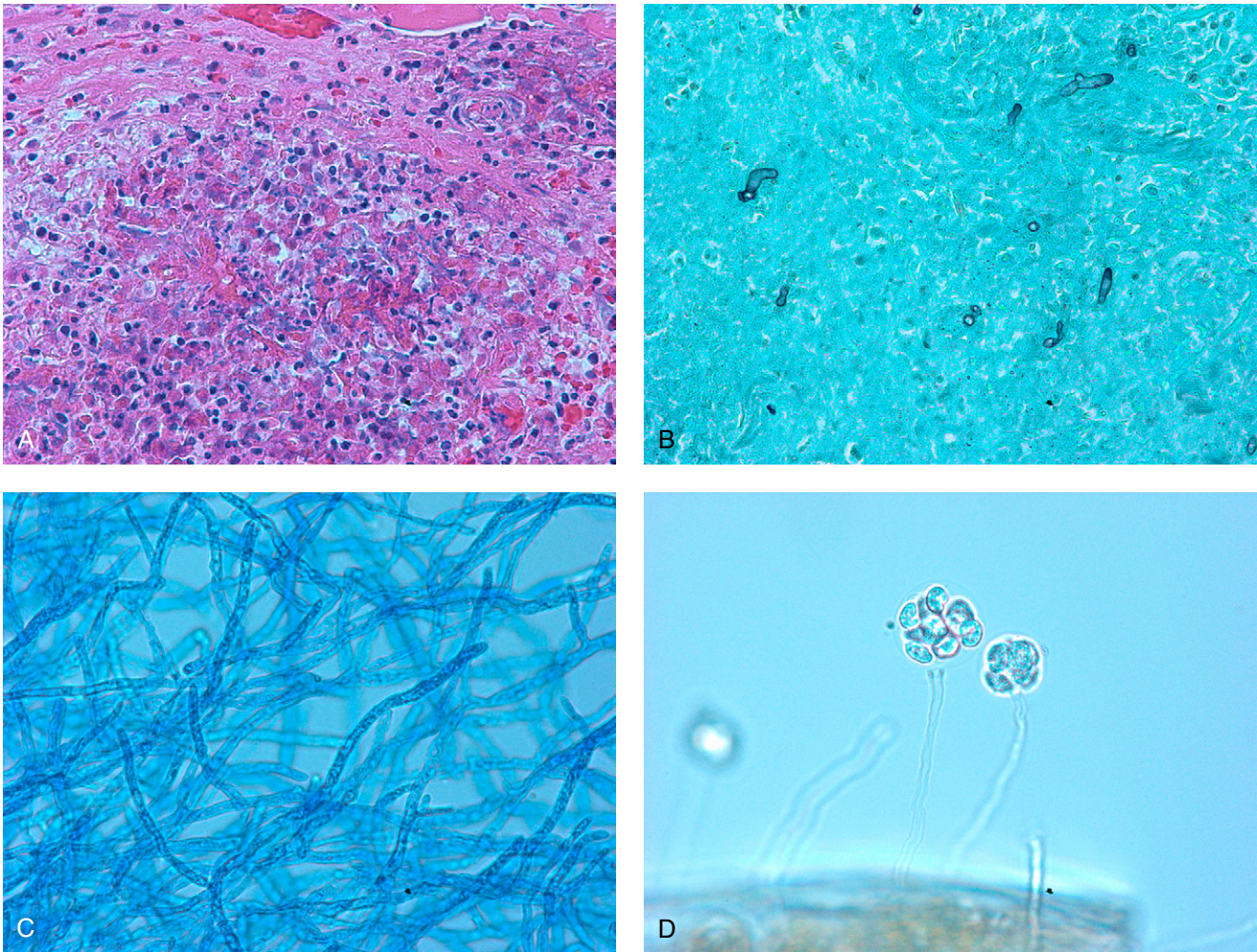
of *P. insidiosum* in 10% KOH can easily be confused with most filamentous fungi.<sup>8</sup> Additionally, primary cultures on 2% Sabouraud dextrose agar are often unsuccessful, which complicates the laboratory diagnosis of the disease. Thus, the alternative use of molecular methodologies has been introduced to diagnose keratitis caused by *P. insidiosum*.<sup>61</sup> Biopsies collected from cases of arterial, subcutaneous, and systemic pythiosis must be transported immediately to the laboratory. For overnight samples sent to reference laboratories, the samples should be shipped in water that contains broad-spectrum antibiotics to inhibit bacterial overgrowth.

Cultures on 2% Sabouraud dextrose agar may develop within the first 24 hours of incubation at 37°C. The presence of whitish-cream submerged colonies is the main feature of *P. insidiosum* in culture. Microscopically, the presence of hyaline, occasionally septate hyphae without fruiting bodies is typical of *P. insidiosum* infections (Fig. 18-4C).<sup>45</sup> The development of oogonia, the teleomorphic stage of the Perosporomycetes, is rare, so the identification of *P. insidiosum* is mainly

based on the development of zooconidia in water cultures (Fig. 18-4D).<sup>38,49,50</sup> However, molecular methodologies should also be used to properly identify this unusual pathogen from cultures and clinical samples.<sup>40,67,68</sup>

Because cultural techniques can be difficult and time-consuming, serologic assays were developed as an aid in the diagnosis of pythiosis in humans and animals. The most commonly used serologic assays for pythiosis are immunodiffusion (ID), enzyme-linked immunosorbent assay (ELISA), Western blot (WB), and latex agglutination (LA).<sup>44,53</sup> Immunodiffusion was one of the first serologic tests developed to detect anti-*P. insidiosum* antibodies in infected hosts.<sup>66,69</sup> Although highly specific, the ID test was insensitive and false negatives, particularly in humans with arterial pythiosis, were frequently encountered.<sup>52</sup> More sensitive assays such as ELISA and WB have been helpful in the early detection of the disease in humans and animals,<sup>43,55,70,71</sup> whereas LA lacked diagnostic specificity.<sup>53</sup> Western blot is considered the most sensitive and specific test for pythiosis. The protein banding patterns in WB are used to





**Figure 18-4** (A) Hematoxylin and eosin-stained histopathologic section of a subcutaneous biopsy from a patient with pythiosis showing ulcerated tissue, microabscesses, necrosis and a mixture of chronic and acute inflammatory infiltrate. The Splendore–Hoeppli phenomenon is observed around the hyphal elements of *Pythium insidiosum*, appearing as empty circular spaces (50 $\times$ ). (B) Gomori methenamine silver-stained section of the same area in panel A. A few transversally and longitudinally short hyphal elements are typically observed in subcutaneous and vascular pythiosis (50 $\times$ ). (C) *P. insidiosum* hyaline, sparsely septate hyphae from slant cultures on 2% Sabouraud dextrose agar (lactophenol blue, 50 $\times$ ). (D) When *P. insidiosum* is placed in water cultures with some cations, the production of sporangia containing numerous asexual biflagellated zooconidia can be observed (50 $\times$ )

specifically link the infection to *P. insidiosum*. However, few laboratories offer these assays for the diagnosis of pythiosis.

Most antifungal drugs target the ergosterol pathway. When this biosynthetic pathway is disrupted, the cytoplasmic membrane breaks, causing leak of the cytoplasm and cell death.<sup>8</sup> *Pythium insidiosum* lacks ergosterol in its cytoplasmic membrane, so it does not properly respond to antifungal drugs. In spite of this, amphotericin B, ketoconazole, terbinafine, and other antifungals, including chemicals such as iodides, have been used, with contradictory results.<sup>45,47,48,57</sup> For instance, two Australian patients with orbital disease were successfully treated with a combination of 0.5 mg/kg/day of amphotericin B + 150mg/kg/day of flucytosine. However, the same combination did not work in Thai and USA patients<sup>45</sup> (unpublished data). Similarly, in the USA a boy with orbital pythiosis responded well to a combination of itraconazole and terbinafine, but this combination did not achieve the same results in other patients.<sup>45</sup> Amputation of

infected limbs, surgical debridement of infected areas, and enucleation of affected eyes is the last-resort treatment in most cases.<sup>44,45</sup> However, surgery has a 40% recurrence rate, which illustrates the difficulties clinicians face with the management of pythiosis.<sup>44</sup>

A non-invasive alternative to surgery is immunotherapy, a unique treatment of pythiosis using proteins extracted from cultures of *P. insidiosum* injected into infected hosts.<sup>45,52,58</sup> This novel therapeutic approach has a 55% cure rate in humans,<sup>53</sup> and in equines it is even more effective, with >70% of injected equines responding favorably.<sup>53</sup> The current therapeutic regimen consists of two injections of *P. insidiosum*'s high molecular weight proteins (0.2  $\mu$ g/ml) 2 weeks apart.<sup>53</sup> The proposed therapeutic mechanism has been linked to the downregulation of the Th2 response to a Th1 reaction, which is somehow triggered by the injected antigens.<sup>53</sup> The Th1 response then stimulates great quantities of IFN- $\gamma$  and IL-2, which in turn trigger the release of T-cytotoxic lymphocytes,

natural killer cells and activated macrophages to the infection site. It is believed that IFN- $\gamma$  downregulates the Th2 response, whereas the cell-mediated reaction apparently kills the hyphal elements of *P. insidiosum* in the infected tissues. The observed side effects related to immunotherapy include pruritic sensation at the infection site, discomfort, fever, and headaches. These side effects disappeared within the first 2–3 days after each injection.<sup>53</sup>

## Rhinosporidiosis

### Etiology

Rhinosporidiosis is an unusual infection of the mucous membranes and, more rarely, of the subcutaneous tissues caused by *Rhinosporidium seeberi*. Although the infection is more commonly diagnosed in Asia, rhinosporidiosis was first reported in South America by Guillermo Rodolfo Seeber in two Argentine patients studied by him in 1896,<sup>72</sup> but he did not name the etiology. The name *Coccidium seeberia* was a binomial given to this pathogen by Seeber's former adviser Wernicke.<sup>73</sup> Unaware of Seeber's report, Minchin and Fantham<sup>74</sup> introduced the binomial *Rhinosporidium kinealyi* based on an identical pathogen from a polypoidal growth in the nose of a male Indian previously studied by O'Kinealy in 1903.<sup>75</sup> However, Seeber in 1912<sup>73</sup> adopted the species name *R. seeberi*, arguing that it had priority over the Minchin and Fantham<sup>74</sup> binomial. In a comprehensive review of the etiologic agent of rhinosporidiosis, Ashworth<sup>73</sup> adopted the name *R. seeberi*.

Ever since the first report of rhinosporidiosis, it was evident that *R. seeberi* resists culture. Thus, its taxonomic as well as its ecologic and epidemiologic features have been largely misunderstood.<sup>76–80</sup> Based on several in vivo characteristics, this pathogen has been traditionally considered both a protozoan and a fungus,<sup>73,76,78,80</sup> and more recently a cyanobacterium, a plant, and/or a carbohydrate waste.<sup>78</sup> However, ultrastructural studies have shown that the spherical phenotypes, including the endoconidia, of *R. seeberi* possessed true nuclei, a fact that undoubtedly placed this pathogen within the eukaryotes.<sup>14,76,80</sup> The molecular studies of Herr et al<sup>81</sup> and Fredericks et al<sup>82</sup> strongly supported early ultrastructural studies. These investigators reported that the 18S small subunit ribosomal DNA sequences (18S SS rDNA), recovered from humans and a dog with rhinosporidiosis clustered as the sister taxon to the homologous sequences of spherical aquatic pathogens of fish and amphibians in the Mesomycetozoa. The genera *Dermocystidium*, earlier described by Carini as morphologically similar to *Rhinosporidium*,<sup>83</sup> and *Sphaerothecum*, for instance, develop uniflagellate zooconidia from the released endoconidia.<sup>10,84</sup> In contrast, *Rhinosporidium* and *Amphibiocystidium*, a skin pathogen of frogs forming sporangia with endoconidia,<sup>85</sup> lack this ability, suggesting that these two microbes have lost this ancestral feature.

The studies of Silva et al<sup>86</sup> further validated the idea that *R. seeberi* is a eukaryotic microbe in the Mesomycetozoa. This new study found that eight internal transcriber spacer (ITS) DNA sequences recovered from eight Indian and Sri Lankan humans, a dog, and two swans with proven rhinosporidiosis consistently grouped within the Mesomycetozoa. The new finding implies that other taxonomic hypotheses regarding this

pathogen may need to be carefully reviewed. The phylogenetic studies of Silva et al<sup>86</sup> also found the presence of species-specific strains in the genus *Rhinosporidium*, a finding that could forecast the existence of novel host-specific species. Adl et al<sup>84</sup> recently reclassified *R. seeberi* in the rank Mesomycetozoa, class Ichthyosporaea, in the family Rhinosporidaeae. Interestingly, as is the case in *R. seeberi*, the mesomycetozoans are also aquatic spherical organisms with previously unknown taxonomic background. Its location at the point where animals and fungi first diverged suggests that this unicellular group of microbes could be one of the ancestors of animals and fungi.

### Distribution and epidemiology

The infections caused by *R. seeberi* have been reported in most tropical, subtropical and temperate areas of the world, except Australia.<sup>77,78,80</sup> The infection is frequently diagnosed in India, Sri Lanka, and Latin America.<sup>7</sup> Although rhinosporidiosis occurs as single cases, sporadic outbreaks have been recorded in humans and swans.<sup>87–89</sup> Little is known about the mechanisms of transmission and the characteristics of the infecting units in nature. It is believed that *R. seeberi* gains entrance to the infected tissues by trauma.<sup>78,80,90</sup> Infected humans and animals have usually associated with wet environments prior to infection.<sup>73,78</sup>

Based on these reports, *R. seeberi* was placed within the hydrophilic pathogens.<sup>19</sup> However, the finding of human rhinosporidiosis in dry areas after sandstorms suggests that *R. seeberi* may also develop resistant conidia in dry environments.<sup>14</sup> The placement of *R. seeberi* within the Mesomycetozoa strongly supports the idea that this pathogen could form motile resistant conidia in wet and dry locations. The endoconidia could remain viable in aquatic as well as in dry ecologic niches for long periods of time, acting as the infectious particles for susceptible hosts.<sup>10</sup> It is unclear whether the released endoconidia remain dormant in nature or have an intermediate host life cycle. However, the absence of intermediate hosts for the other members of the Mesomycetozoa does not support this belief.

### Pathogenesis and clinical features

*Rhinosporidium seeberi*, as in the other mesomycetozoans, develops sporangia (cysts) with endoconidia. The endoconidia are subsequently released from mature sporangia in the infected host tissues and to the environment. Susceptible hosts may then acquire the infection after traumatic implantation of the resistant conidia. Unfortunately, experimental infection in animals has so far been unsuccessful.<sup>91</sup> Silva et al<sup>86</sup> recently found host-specific strains in the genus *Rhinosporidium*, which may explain the lack of success in a mouse model experimentally challenged with clinical samples from human rhinosporidiosis.

In endemic areas the disease is more frequently diagnosed in males than females; this includes children and adults.<sup>77,78,80</sup> The infection is not life threatening. Painless obstructive polypoidal masses that easily bleed are commonly found in the eyes, nostrils, pharynx, nasopharynx, and less frequently in other anatomic areas.<sup>73,76–78,80</sup> Unusual cases involving internal organs have been sporadically reported.<sup>78</sup> In the infected eye, friable pedunculated polyps are commonly diagnosed. Tearing, conjunctival irritation, and photophobia are the main clinical features<sup>92,93</sup> (Fig. 18-5A). Polypoidal masses in the pharynx



and nasopharynx may lead to swallowing, breathing and food intake difficulties.<sup>73,74,78,80</sup> Rhinosporidial polyps in the nose are characterized by nasal bleeding and obstruction.<sup>15,78</sup> Rhinosporidiosis of the genitalia, skin, and internal organs is rare.<sup>94</sup> Infections caused by *R. seeberi* have to be differentiated from bacterial and fungal infections of the infected sites as well as neoplasias and other mucosal and skin diseases.<sup>78</sup> There is no evidence of either zoonotic or person-to-person transmission of the infection.

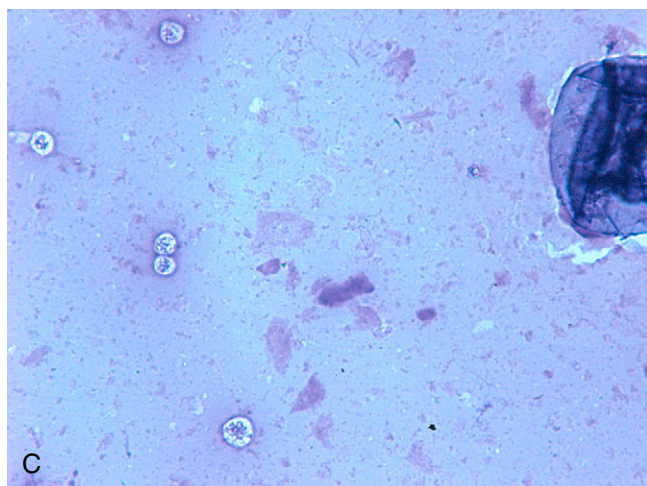
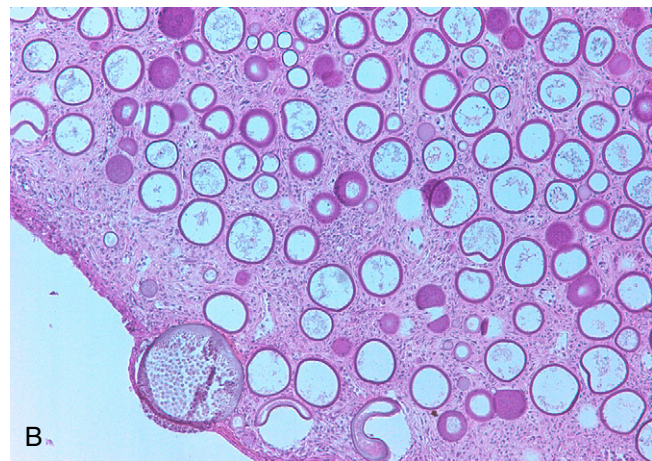
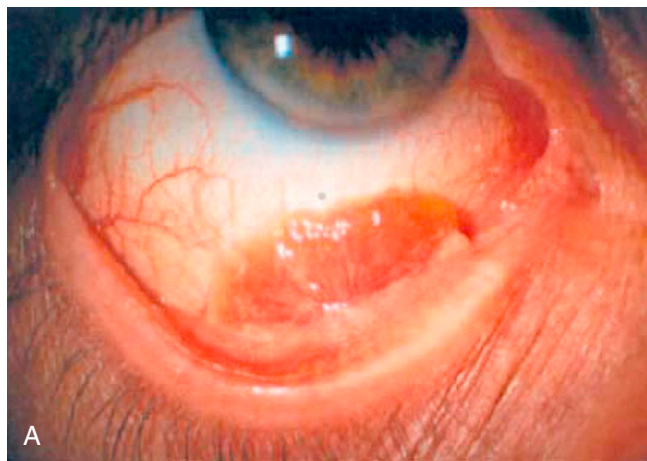
## Pathology, laboratory diagnosis and treatment

The parasitic spherical phenotypes of *R. seeberi* are readily observed in histopathologic sections of infected tissues. The immature and mature sporangia with endoconidia are well stained by H&E. However, they also stain well with PAS and silver stains, a feature used by some to classify *R. seeberi* within the fungi.<sup>76,78,80</sup>

In infected tissue *R. seeberi* develops a complex life cycle. It starts with the enlargement of non-flagellated 5–20  $\mu\text{m}$  diameter endoconidia released from mature sporangia through programmed pore formation.<sup>95</sup> The released endoconidia enlarge to become immature 40–60  $\mu\text{m}$  diameter sporangia with a thick cell wall and no endoconidia. These forms evolve

to become mature sporangia with a thin cell wall and hundreds of endoconidia. The mature sporangia may reach 100–500  $\mu\text{m}$  in diameter, a feature that contrasts with the smaller spherical parasitic phenotype of *Coccidioides* spp. The mature sporangia have been reported to be transported from the internal infected tissues to the surface of the polyps by a transepidermal mechanism.<sup>78</sup> The finding of mature sporangia with the pore pointed to the outside of the tissues at the edges of the epidermal layers may suggest that the proposed transepidermal mechanism is an evolutionary strategy to release the endoconidia back to the pathogen environmental original stage.

The infected tissue shows hyperplasia with a chronic granulomatous inflammatory response and a marked vascularity usually associated with hemorrhagic polyps after trauma or surgical intervention. Lymphocytes, plasma cells, macrophages, giant cells and, more rarely, eosinophils are usually found around the spherical phenotypes of *R. seeberi*.<sup>76,80,87</sup> Large numbers of sporangia at different stages of development are a main feature of the histopathologic tissue sections (Fig. 18-5B). Active macrophages are also seen with engulfed endoconidia. However, due to the overwhelming quantities of endoconidia, sufficient numbers evade elimination to become immature and mature sporangia, thus ensuring pathogen survival. The presence of U-shaped collapsed sporangia is common in most histopathologic preparations (Fig. 18-5B).



**Figure 18-5** Clinical, histopathologic and histologic features of *Rhinosporidium seeberi*. (A) Typical ocular rhinosporidiosis involving the bulbar conjunctiva (courtesy of the *Canadian Journal of Ophthalmology* from Harissi-Dagher et al. *Can J Ophthalmol* 41:226-229, 2006). (B) The hematoxylin and eosin-stained histopathologic section shows sporangia at different stages of development from a Venezuelan patient with ocular rhinosporidiosis (20 $\times$ ). Transepidermal relocation of mature sporangia with endoconidia to the epidermal layers is seen. Some U-shaped immature sporangia are also observed (40 $\times$ ). (C) Giemsa-stained smear from a case of nasal rhinosporidiosis. Note the presence of a mature collapsed sporangium (right section of the panel) as well as several released endoconidia (round small bodies) (50 $\times$ ).



The diagnosis of rhinosporidiosis in the clinical laboratory is based on wet preparations of the infected polyps and less frequently on smears stained with Giemsa and Wright<sup>96</sup> (Fig. 18-5C). A report on the value of smears for histologic evaluation in suspected cases of rhinosporidiosis was recently published.<sup>97</sup> The presence of large spherical sporangia with endoconidia in 10% KOH has to be differentiated from similar structures observed in cases of coccidioidomycosis. In addition, subcutaneous myospherulosis first described in the earliest 1970s,<sup>98</sup> with spherical masses that do not take the dyes of fungal stains, has to be differentiated from the spherical phenotypes of *R. seeberi*. Cultures have to be performed to rule out other etiologies. Serologic assays for rhinosporidiosis are not available.

The treatment of rhinosporidiosis is difficult. The most common procedure is surgical removal of infected polyps.<sup>36,78,80</sup> However, profuse bleeding and recurrence of the polyps are common. Antifungal drugs do not have any effect on *R. seeberi*.<sup>80</sup> Nevertheless, reports of cure using amphotericin B have been published in cases of eye infection by *R. seeberi*.<sup>78</sup> However, these claims have been questioned due to the finding of spontaneous regression of some rhinosporidial lesions in previously infected hosts.<sup>77</sup>

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# Fungal infections in the patient with human immunodeficiency virus infection

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Fungi are important pathogens in patients with the acquired immunodeficiency syndrome (AIDS) due to human immunodeficiency virus (HIV) infection. This chapter provides an overview of fungal infection in the setting of HIV, reviewing the epidemiology, clinical features, and treatment of mucosal candidiasis, cryptococcosis, histoplasmosis, coccidioidomycosis, penicilliosis, and some less common invasive fungal infections seen in this population. A discussion of drug interactions associated with co-administration of antifungal medications and antiretroviral agents is included. Finally, this chapter addresses the prevention of fungal diseases in the HIV-infected patient.

The importance of fungal diseases among patients with HIV infection was recognized in the early days of the AIDS epidemic. Fungal infections were reported in the first patients described with a “new acquired cellular immunodeficiency” in 1981.<sup>1,2</sup> Soon thereafter, the Centers for Disease Control and Prevention (CDC) proposed a case definition for AIDS, which included cryptococcosis, esophageal candidiasis, and invasive forms of candidiasis, aspergillosis, and zygomycosis among the opportunistic infections indicative of immunodeficiency.<sup>3</sup> The current case definition includes candidiasis of the bronchi, trachea or lungs; esophageal candidiasis; disseminated or extrapulmonary coccidioidomycosis; extrapulmonary cryptococcosis; and disseminated or extrapulmonary histoplasmosis.<sup>4</sup>

*Pneumocystis jiroveci*, a unicellular fungus which was classified previously as a protozoan, causes pneumocystis pneumonia (PCP) in patients with AIDS. The important topic of PCP is covered in Chapter 17 of this book.

## Risk of fungal infection in the patient infected with HIV

The risk of a fungal infection developing depends primarily on the following factors: (1) the severity of impairment of cell-mediated immunity, (2) recent or current use of an antifungal medication, (3) the risk of exposure, and (4) neutropenia, which relates to invasive candidiasis and aspergillosis. Impairment of cell-mediated immunity predisposes to cryptococcosis, disseminated histoplasmosis, coccidioidomycosis, and mucocutaneous candidiasis.<sup>5</sup> In clinical practice the CD4+ T lymphocyte percentage or absolute count indicates the degree

of immunosuppression which HIV infection causes over time. Generally, patients with the lowest CD4+ counts are at greatest risk of fungal infection.

The management of HIV-infected patients has improved greatly over the last decade; in particular, great strides have been made in the field of antiretroviral therapy. Several efficacious antiretroviral regimens are available, many have been simplified with lower pill burdens and fewer daily doses. Initiation of highly active antiretroviral therapy (HAART) before depletion of CD4+ lymphocytes can significantly delay the deterioration of cell-mediated immune function which would otherwise occur. In the AIDS patient who has already experienced a substantial decline in the number of CD4+ T lymphocytes, HAART may be associated with reconstitution of immune function. Since the introduction of HIV-protease inhibitors in the mid 1990s and their subsequent use in combination with nucleoside reverse transcriptase inhibitors, morbidity and mortality due to AIDS have declined.<sup>6</sup> Data suggest that the use of HAART is associated with a reduction in the risk of opportunistic infections, including those which are due to fungi.

Unfortunately, not all HIV-infected patients are aware of their infection until they have AIDS, while some others who receive antiretroviral therapy do not respond optimally. Among these individuals with low CD4+ counts, fungal infections continue to occur. While standard care for the HIV-infected patient includes primary prophylaxis against PCP, *Mycobacterium avium* complex infection and toxoplasmosis, primary prophylaxis for fungal infection is not recommended.<sup>7</sup> The potential role of antifungal agents in the primary prevention of fungal diseases is discussed later in this chapter.

## Fungal diseases of particular importance in the patient infected with HIV

### Candidiasis

The degree of immunosuppression, as inferred from the CD4+ count, influences the risk, severity, and anatomic location of mucosal candidiasis.<sup>8,9</sup> Oropharyngeal candidiasis (OPC)

becomes more common when the count falls below 300/mm<sup>3</sup> and esophagitis occurs when the count is less than 100/mm<sup>3</sup>.<sup>8,10</sup> The prevalence of OPC and esophageal candidiasis began decreasing after the introduction of protease inhibitors in 1996; patients whose CD4+ counts increase in response to HAART have lower risk.<sup>11</sup> Episodes continue to occur among patients who do not experience significant increases in their CD4+ count. The severity of CD4+ T lymphocyte depletion is not the sole determinant of risk, however, as recent data suggest an independent, inverse relationship between plasma HIV viral load and the prevalence of OPC and esophageal candidiasis.<sup>12</sup> The protective mechanism may involve a direct effect, as HIV-protease inhibitors have been shown to interfere with adherence of *Candida albicans* to epithelial cells in vitro.<sup>11</sup>

Despite the high frequency with which *Candida* species colonize and cause disease on the mucosal surfaces of patients with HIV, systemic candidiasis is a relatively uncommon problem in this population. Risk factors similar to those seen in other patient groups predispose patients with HIV to candidemia: neutropenia with or without preceding cytotoxic chemotherapy, the presence of an intravenous catheter, and intravenous hyperalimentation.

*Candida* species colonize the gastrointestinal tract and in most cases, the strain causing disease is derived from the patient's own flora. *C. albicans* remains the most common species to cause disease at any mucosal site but *C. glabrata*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis*, either alone or in combination with *C. albicans*, are being recognized increasingly as pathogens, especially among patients with advanced AIDS who take azole antifungal agents chronically.<sup>11,12</sup>

### Oropharyngeal candidiasis

Some of the first patients reported with AIDS had OPC, which is the most common oral infection observed in patients with HIV.<sup>1,2</sup> In the absence of antifungal therapy, almost all patients with AIDS in the pre-HAART era developed OPC eventually.<sup>9</sup> The occurrence of OPC may be a sentinel event, preceding other opportunistic infections and predicting progression to AIDS.<sup>11,13</sup> In fact, the occurrence of OPC is an indication to initiate primary prophylaxis against PCP, regardless of the CD4+ count.<sup>7</sup> In a prospective trial evaluating the efficacy of fluconazole for the prevention of mucosal candidiasis among women with CD4+ counts <300/mm<sup>3</sup>, the incidence of OPC in the placebo group over a median follow-up period of 29 months was 42%.<sup>10</sup> More recent data suggest that antiretroviral therapy that includes a protease inhibitor is associated with reduced susceptibility to new and relapsed OPC.<sup>14,15</sup>

Four types of oropharyngeal disease are recognized: pseudomembranous (thrush), erythematous (atrophic), hyperplastic (candidal leukoplakia), and angular cheilitis. Pseudomembranous OPC variably causes burning pain, dysphagia, and altered taste or it may cause no symptoms. White or tan plaques, which are easily removable with a tongue blade, may be present on the tongue, palate, buccal mucosa, gums, and pharyngeal mucosa. The patient with atrophic OPC may complain of mouth soreness; examination reveals erythematous patches on the palate, buccal mucosa, and dorsum of the tongue. Hyperplastic OPC, which often causes no symptoms, is associated with thin, firmly adherent white patches on the commissures, buccal mucosa, palate, and tongue. Inflammation at the angles of the mouth, angular cheilitis, causes

burning pain and soreness. The diagnosis of OPC is based on the gross appearance of the lesions, sometimes supplemented by microscopic examination of potassium hydroxide (KOH)-treated scrapings, which contain pseudohyphae and budding yeast. Culture alone is not diagnostic because it does not differentiate colonization without disease from disease. Culture is reserved for refractory cases when the goal is to identify non-*albicans* species, such as *C. krusei* and *C. glabrata*, which are likely to be resistant to azole antifungals.

Options for initial therapy of OPC include a topical regimen with nystatin suspension, nystatin pastilles or clotrimazole troches, or systemic treatment with fluconazole or itraconazole suspension.<sup>16</sup> Therapy is given for 7–14 days. Clinical cure rates are greater with 100 mg/day of fluconazole than with 500,000 units four times per day of nystatin liquid.<sup>17</sup> Comparative trials evaluating clotrimazole troches and fluconazole have yielded inconsistent results.<sup>18,19</sup> Overall, systemic therapy is associated with higher mycologic cure rates and lower relapse rates than topical therapy, and topical therapy requires multiple daily doses, which sometimes limits patient acceptance.

One approach to mild disease is to treat with 10 mg clotrimazole troches, dissolved in the mouth 4–5 times per day, reserving systemic therapy for initial treatment of more severe disease and for refractory disease. Itraconazole suspension, which is better absorbed than capsules, at a dose of 200 mg/day for 14 days is at least as effective as 100 mg/day of oral fluconazole for 14 days (97% vs 87% clinical response rates for itraconazole suspension and fluconazole, respectively).<sup>20</sup> Posaconazole, a newer broad-spectrum triazole, is as effective as fluconazole for the treatment of OPC.<sup>21</sup> When efficacy, side effects, the potential for drug interactions, cost, and patient acceptance are considered together, oral fluconazole is the preferred first-line therapy for OPC in the patient with HIV.

### Esophageal candidiasis

Approximately 10–20% of patients with AIDS developed esophageal candidiasis in the pre-HAART era.<sup>22</sup> Although esophageal candidiasis remains one of the most common opportunistic infections seen with AIDS, its incidence has declined since the mid 1990s. From 1996 to 1998, the case rate was 4.3/100 person-years among patients enrolled in the Adult and Adolescent Spectrum of Disease Project of the CDC.<sup>23</sup> During that time, the incidence of candidal esophagitis decreased almost 17% per year. Studies performed in other populations of AIDS patients also indicate a significant decrease in the incidence of esophageal candidiasis in conjunction with the use of HAART.<sup>24–26</sup> In a multicenter European study, the incidence of esophageal candidiasis declined by over 90% from 1995 to 2004, during which time the concomitant use of antifungal drugs also declined significantly.<sup>26</sup>

Esophageal candidiasis is the most frequent cause of dysphagia and odynophagia in patients with AIDS.<sup>27–29</sup> Most patients with OPC and esophageal symptoms have esophageal candidiasis but not all patients with esophageal candidiasis have OPC.<sup>27</sup> That is, the absence of OPC does not exclude mucosal candidiasis as the cause of esophageal symptoms. Empiric systemic antifungal therapy is an efficacious, safe, and cost-effective initial step in the management of the HIV-infected patient with esophageal symptoms.<sup>29</sup> Patients who respond to empiric therapy are diagnosed presumptively with esophageal candidiasis and receive a full course of therapy.

Patients who do not respond to therapy (or those who develop esophageal symptoms while receiving antifungal therapy) require further diagnostic evaluation to identify other potential causes such as herpes simplex virus, cytomegalovirus or idiopathic ulceration. Direct visualization of the mucosa with fiberoptic endoscopy, which is more sensitive for the diagnosis of esophageal candidiasis than barium esophagography, is the diagnostic procedure of choice.<sup>27</sup> Up to 50% of HIV-infected patients with esophageal symptoms are infected with more than one pathogen, and endoscopy offers the best opportunity for a complete diagnosis.<sup>27,30</sup>

Fluconazole is the drug of choice for empiric therapy and for the treatment of proven esophageal candidiasis. Typically, 200 mg of fluconazole is given on the first day, followed by 100 mg/day to complete 14–21 days. For the patient who is unable to swallow, initial therapy is given intravenously; otherwise oral administration is preferred. Itraconazole oral suspension (100–200 mg/day) was associated with clinical success comparable to that achieved with fluconazole at the same doses in a randomized double-blind trial.<sup>31</sup> Voriconazole, 200 mg twice daily, was found to be at least as effective as fluconazole for esophageal candidiasis in a prospective trial of immunocompromised patients, most of whom had AIDS.<sup>32</sup> Multiple prospective investigations, which included exclusively or predominantly AIDS patients, have shown that all three currently available echinocandins (caspofungin, micafungin, and anidulafungin) are as effective as fluconazole.<sup>33–35</sup> However, fluconazole remains the preferred option for initial therapy because it is cheaper than the echinocandins and because the echinocandins require intravenous dosing.

### Vulvovaginal candidiasis

Whether vulvovaginal candidiasis occurs more frequently among HIV-infected women than it does among those without HIV is uncertain.<sup>36,37</sup> In a cross-sectional analysis, vulvovaginal candidiasis was equally prevalent among HIV-infected and HIV-uninfected women (9% in both groups).<sup>36</sup> In addition, the prevalence of disease did not increase with decreasing CD4+ counts, so unlike OPC and esophagitis, vulvovaginal candidiasis does not indicate progressive immune dysfunction.<sup>36</sup> Signs and symptoms of vulvovaginal candidiasis include pruritus, dyspareunia, a white cheesy discharge, dysuria, and vulvar erythema. The presence of yeast forms in a microscopically examined KOH preparation confirms the diagnosis. A single 150 mg dose of oral fluconazole and a 7-day course of once-daily clotrimazole vaginal suppositories each achieved clinical cure rates of ~75% in a comparative trial among HIV-negative women.<sup>37</sup> Clinical experience suggests that both topical therapy and systemic fluconazole are also effective for the treatment of HIV-infected women. Itraconazole oral suspension at 200 mg twice daily for 1 day or 200 mg/day for 3 days is another option for vulvovaginal candidiasis.<sup>38</sup>

### Management of relapsed and refractory mucosal candidiasis

Initial treatment of mucosal candidiasis is usually successful but relapse of OPC and esophageal candidiasis is almost universal among patients without reconstituted immunity after antifungal therapy is discontinued.<sup>39,40</sup> Fluconazole, when taken continuously on a daily basis or once weekly, effectively decreases the number of recurrences of OPC in patients with

HIV.<sup>40,41</sup> However, many clinicians are reluctant to prescribe continuous fluconazole for secondary prophylaxis because:

- future episodes of mucosal candidiasis can be diagnosed and treated with relative ease
- most cases of OPC are associated with minimal morbidity
- adverse drug reactions may occur
- long-term use of fluconazole predisposes to infection with azole-resistant strains of *Candida*.

The last issue was studied in a randomized trial comparing continuous with intermittent fluconazole for patients who had been treated successfully for an episode of OPC.<sup>42</sup> Continuous therapy was associated with a relapse rate of 0 episodes per year; intermittent therapy was associated with a rate of 4.1 episodes per year ( $P < 0.001$ ).<sup>42</sup> Strains with in vitro resistance to fluconazole (minimum inhibitory concentration (MIC)  $> 16 \mu\text{g/ml}$  in this study) appeared in 56% and 46% of patients in the continuous and intermittent groups, respectively.<sup>42</sup> This difference was not statistically significant and importantly, all but two of the patients with resistant strains were treated successfully with higher doses of fluconazole. Given these data, it is reasonable to reserve continuous secondary prophylaxis for patients with a history of frequent recurrences and to treat those with infrequent recurrences with intermittent courses of fluconazole as needed.

Management of disease that is refractory to fluconazole is another clinical challenge. Fluconazole failure is almost always associated with a strain of *Candida* that possesses in vitro resistance to fluconazole but the converse is not always true; some OPC caused by strains that show in vitro resistance to fluconazole will respond to the drug.<sup>43</sup> Risk factors for fluconazole-resistant candidiasis in the setting of HIV include advanced immunosuppression as assessed by CD4+ count and greater median duration of exposure to fluconazole.<sup>44,45</sup> Options for the treatment of fluconazole-refractory OPC include topical therapies such as clotrimazole troches, oral amphotericin B (1 ml four times a day of the 100 mg/ml suspension) or gentian violet.<sup>43</sup> Systemic alternatives for refractory OPC and esophageal candidiasis include higher doses of fluconazole (400–800 mg once or twice a day), itraconazole suspension (200 mg twice daily), voriconazole (200 mg twice daily), intravenous caspofungin (50 mg/day), and intravenous amphotericin B (0.3–0.7 mg/kg/day).<sup>16</sup>

### Cryptococcosis

In the pre-HAART era disseminated cryptococcosis affected 5–10% of people with AIDS in developed countries, making it the most common life-threatening fungal infection in this population.<sup>46</sup> In 40% of cases, cryptococcosis is the patient's first AIDS-defining condition.<sup>46</sup> The CD4+ count is almost always less than  $100/\text{mm}^3$  and is usually less than  $50/\text{mm}^3$  at the time of diagnosis.<sup>47</sup> Geography and race appear to influence the risk of cryptococcosis among patients with HIV in the United States, with the highest rates of disease existing among African-Americans living in the southeast.<sup>48</sup> More recent data suggest that the association between risk and race is likely due to socioeconomic disadvantage among African-Americans and lack of access to healthcare, more specifically lack of access to HAART.<sup>49</sup> Surveillance data from Europe and the United States indicate that the incidence of cryptococcosis has



declined significantly since the introduction of HAART.<sup>49-51</sup> In France, the incidence declined by 46% in the post-HAART era (1997–2000) compared to the pre-HAART era of 1985–1996.<sup>51</sup> From the early 1990s to 2000, case rates fell from 66 per 1000 persons with AIDS to 7 per 1000 in Atlanta and from 24 per 1000 to 2 per 1000 in Houston.<sup>49</sup> The majority of cases occurred among patients who were not receiving HAART.

Subacute meningitis and meningoencephalitis are the most common manifestations of cryptococcosis in the patient with AIDS. Typically, patients seek medical attention after about 2–4 weeks of illness.<sup>46</sup> Headache, fever, malaise, visual changes, nausea, vomiting, and respiratory complaints (due to concomitant lung infection) are common symptoms.<sup>52-54</sup> Mental status changes range from lethargy, decline in cognitive function and memory loss, to frank obtundation and coma. Fever is the most consistent physical finding. Less than 40% of HIV-infected patients with cryptococcal meningitis have photophobia or neck stiffness, and focal neurologic deficits are detected in less than 20% of patients.<sup>46,52-54</sup> Unlike immunocompetent patients with pulmonary cryptococcosis, almost all patients with AIDS and pulmonary cryptococcosis have disseminated disease, including meningitis.<sup>55</sup> Bilateral alveolar and interstitial opacities are the most typical radiographic findings.<sup>55</sup> Disseminated cryptococcosis may involve virtually any organ, including the skin, with lesions resembling molluscum contagiosum, and the prostate, which may serve as a nidus for persistent infection.<sup>46</sup>

From 95% to 99% of AIDS patients with cryptococcal meningitis have a serum cryptococcal antigen titer of greater than 1:8, and for the patient with fever, this assay is an excellent screening test; a negative result essentially excludes disseminated cryptococcosis.<sup>46</sup> When the clinical scenario suggests cryptococcosis or when the serum cryptococcal antigen assay is positive, evaluation should include brain imaging to exclude the possibility of a mass lesion that would be a contraindication to lumbar puncture, followed by lumbar puncture if no mass is seen. Imaging is not performed to detect a cryptococcoma, which is rare in the AIDS patient, but rather to exclude a mass caused by another process such as toxoplasmosis or lymphoma. The concentrations of glucose and protein in the cerebrospinal fluid (CSF) may be normal in the AIDS patient with cryptococcal meningitis, and CSF pleocytosis may be absent. Therefore, a normal CSF formula does not exclude the diagnosis. However, the organism load is typically very high and therefore, the CSF cryptococcal antigen assay is virtually always positive, and the India ink stain is positive in about 75% of patients.<sup>46</sup> The gold standard for diagnosis of cryptococcosis is growth of *Cryptococcus neoformans* in culture from a normally sterile body fluid, usually CSF or blood, or consistent histopathologic findings. Blood cultures grow the fungus in about 75% of patients with AIDS-associated cryptococcal meningitis.

Treatment of cryptococcal meningitis in patients with AIDS can be divided into three phases: induction, consolidation, and maintenance or chronic suppression. The Mycosis Study Group (MSG) of the National Institute of Allergy and Infectious Diseases (NIAID) and the AIDS Clinical Trials Group (ACTG) performed a prospective, randomized, double-blind trial comparing induction therapy with 2 weeks of amphotericin B 0.7 mg/kg/day alone vs amphotericin B 0.7 mg/kg/day plus 5-flucytosine (5FC) 100 mg/kg/day, and consolidation

therapy with 8 weeks of oral fluconazole vs itraconazole, each at 400 mg/day.<sup>54</sup> The addition of 5FC was associated with a trend toward faster sterilization of the CSF and a decreased risk of later relapse but not decreased mortality.<sup>54,56</sup> In the consolidation phase of the study, there was a trend toward better clinical outcomes in the fluconazole group.<sup>54</sup> Itraconazole 400 mg/day seems to be a suitable alternative for consolidation therapy for patients who cannot take fluconazole. There continues to be interest in using fluconazole as initial therapy, and an all-oral regimen of fluconazole and 5FC has been successful in select patients.<sup>57</sup>

Lipid formulations of amphotericin B have been studied in AIDS-associated cryptococcal meningitis but the data to support the routine use of these agents are not robust.<sup>58-60</sup> For the patient with impaired renal function, use of one of the lipid formulations in place of conventional amphotericin B is appropriate. The optimal dose of any lipid preparation has not been determined but liposomal amphotericin B 4 mg/kg/day is recommended.<sup>61</sup>

Factors predictive of higher mortality in the AIDS patient with cryptococcal meningitis include abnormal mentation on presentation, a high CSF cryptococcal antigen titer (>1:1024), and a low CSF white cell count (<20/mm<sup>3</sup>).<sup>53</sup> Elevation of intracranial pressure, as measured with manometry during lumbar puncture, has been associated with catastrophic neurologic complications, including visual loss and death.<sup>62</sup> In addition to antifungal therapy, acute management of cryptococcal meningitis should include lowering the intracranial pressure with repeated lumbar punctures or, if necessary, with placement of a lumbar drain or ventricular shunt. The overall mortality in the MSG trial was 6%, which is substantially lower than that of earlier studies.<sup>54</sup> A higher amphotericin B dose and more careful management of increased intracranial pressure may have contributed to the better outcomes achieved in this study.

Because of high relapse rates, chronic suppressive therapy is given after induction and consolidation therapy.<sup>63</sup> Fluconazole 200 mg/day is highly effective for preventing relapse and in separate trials was found to be superior to weekly amphotericin B and itraconazole 200 mg/day.<sup>56,64</sup> For patients who cannot take fluconazole, itraconazole 400 mg/day may be the best alternative for chronic suppression of cryptococcosis. For patients who are not receiving HAART, duration of maintenance therapy is life-long. Data from several small prospective and retrospective studies suggest that patients who have immune reconstitution in response to HAART may safely discontinue maintenance antifungal therapy.<sup>65,66</sup> Discontinuation is reasonable for the patient who has completed the induction and consolidation phases of treatment, who has no symptoms due to cryptococcosis, and who has had a CD4+ count >100–200 cells/mm<sup>3</sup> for more than 6 months after starting HAART.<sup>38</sup>

## Histoplasmosis

The dimorphic fungus *Histoplasma capsulatum* causes histoplasmosis, which is endemic in certain regions of North America and Latin America, including the Ohio and Mississippi River valleys of the United States. *H. capsulatum* var. *duboisii* causes disease in Africa. Infection occurs by means of inhalation of airborne microconidia with conversion to yeast forms in the lung and subsequent hematogenous dissemination. Bird and bat droppings enhance the growth of the mycelial phase

of *H. capsulatum*, and soil in close proximity to chicken coops and starling roosts and within caves inhabited by bats may contain high numbers of infectious spores.<sup>67</sup> In a prospective study from an endemic area, exposure to chicken coops was associated with an increased risk of histoplasmosis among HIV-infected patients.<sup>68</sup> Both primary infection and reactivation of previously acquired *H. capsulatum* appear to contribute to new cases of histoplasmosis among HIV-infected patients who reside in endemic areas.<sup>68</sup> Molecular investigation supports reactivation as the mechanism of disease among patients who develop histoplasmosis while residing in non-endemic areas.<sup>69</sup>

In the pre-HAART era, histoplasmosis occurred in 2–5% of AIDS patients living in endemic areas but much higher rates were reported during outbreaks in certain locales, including Indianapolis where ~27% of AIDS patients were diagnosed with histoplasmosis between 1980 and 1989.<sup>70</sup> The CD4+ count is usually <100/mm<sup>3</sup> (median ~50/mm<sup>3</sup>) when disseminated histoplasmosis is diagnosed.<sup>71,72</sup> Among patients residing in endemic regions, the risk of histoplasmosis increases when the CD4+ count drops below 150/mm<sup>3</sup>.<sup>68</sup> Among patients enrolled in a case-control study from 1996 to 1999, receipt of antiretroviral therapy was independently associated with decreased risk of histoplasmosis.<sup>72</sup> As with cryptococcosis, disseminated histoplasmosis disproportionately affects those people with AIDS who lack access to medical care and HAART.<sup>72</sup>

Approximately 95% of patients with HIV infection and histoplasmosis have progressive disseminated disease.<sup>70</sup> Clinical manifestations typically develop over 1–3 months and reflect distribution of *H. capsulatum* throughout reticuloendothelial tissues. Fever, weight loss, hepatosplenomegaly, and lymphadenopathy are characteristic features. Diffuse pneumonitis causes respiratory symptoms in approximately 50% of patients. From 10% to 20% of patients have central nervous system manifestations, which include encephalitis, brain mass lesions, and meningitis.<sup>70,73,74</sup> Other sites of disease include the skin and gastrointestinal tract, including the oropharynx, where mucosal ulcerations may occur.<sup>75</sup> From 10% to 20% of patients have sepsis with multiorgan failure and disseminated intravascular coagulation.<sup>70,76</sup>

*H. capsulatum* can grow in cultures of bone marrow, blood, and other specimens as suggested by clinical manifestations (e.g., bronchoalveolar lavage fluid, CSF, tissue biopsies) in approximately 85% of patients with AIDS and disseminated histoplasmosis. Bone marrow gives the highest yield (75%); up to 70% of patients have positive blood cultures if the lysis centrifugation technique is used.<sup>77</sup> Cultures take up to 4 weeks to complete and therefore depending solely on culture will delay diagnosis. Histopathologic examination of biopsy material is less sensitive than culture, with positivity rates of 40–50%.<sup>77</sup> Detection of *H. capsulatum* antigen in body fluids is a reliable and rapid method of diagnosis.<sup>78</sup> With urine, the sensitivity of this assay is 95% among HIV-infected patients with disseminated histoplasmosis; with serum, the sensitivity is 85%.<sup>77</sup> Antigen detection has a specificity of 98%.<sup>78</sup> This test is performed at MiraVista Diagnostics, Indianapolis, IN.

Treatment of disseminated histoplasmosis in the setting of AIDS is divided into the phases of induction and chronic maintenance to prevent relapse. Amphotericin B 0.7–1 mg/kg/day or liposomal amphotericin B 3–4 mg/kg/day is recommended for initial therapy of moderate or severe histoplasmosis,

including central nervous system disease.<sup>38,79</sup> From a practical perspective, this includes all patients who are sufficiently ill to require hospitalization. A randomized trial found liposomal amphotericin 3 mg/kg/day to have overall better clinical efficacy than conventional amphotericin B 0.7 mg/kg/day.<sup>80</sup> Infusion-related toxicities and nephrotoxicity were less common in the group that received the liposomal product.<sup>80</sup> About 80% of patients who are treated with amphotericin B ultimately achieve clinical remission, with satisfactory clinical responses occurring in as little as 3 days in patients with moderate manifestations to 14 days in patients with severe disease. After the patient receiving amphotericin B becomes afebrile, is no longer requiring intensive support and is able to take oral medications, a switch can be made to oral itraconazole 200 mg twice a day to complete 12 weeks of induction therapy.<sup>79</sup> Mildly ill patients without central nervous system disease may be treated initially with oral itraconazole 200 mg three times per day for 3 days, followed by 200 mg twice daily for 12 weeks.<sup>81</sup> Fluconazole 800 mg/day is less effective (74% response rate) and should be used only in patients who cannot take itraconazole.<sup>82</sup>

Without maintenance therapy or immune reconstitution, 35–80% of AIDS patients with disseminated histoplasmosis will experience relapse.<sup>70,75</sup> Although some patients require continuation of itraconazole 200 mg twice daily for effective suppression, the clinically stable patient with low urine *Histoplasma* antigen levels may be switched to itraconazole 100 mg twice daily after completing 12 weeks of induction therapy.<sup>7,71</sup> Overall, lifelong itraconazole 200–400 mg/day prevents relapse in ~90% of patients.<sup>71</sup> Amphotericin B 50–100 mg, given weekly or twice weekly, prevents relapse in 80–95% of patients who respond to induction therapy.<sup>70,83</sup> However, the need for intravascular access, problems associated with indwelling intravascular devices, and drug toxicity may complicate chronic amphotericin B therapy.<sup>71,83</sup> Fluconazole 400 mg/day is less effective than either itraconazole or amphotericin B for maintenance therapy.<sup>82</sup> A prospective observational study suggested that antifungal therapy can be stopped safely in patients who have received at least 12 months of maintenance therapy, have been on HAART for at least 6 months, and have a CD4+ count >150 cells/mm<sup>3</sup>.<sup>84</sup> However, the number of patients evaluated thus far is not sufficient to justify a recommendation to discontinue secondary prophylaxis in this population.<sup>38</sup>

## Coccidioidomycosis

Infection with *Coccidioides immitis* causes coccidioidomycosis, which is endemic in the southwestern United States, northern Mexico, and portions of Central and South America. A second species of *Coccidioides*, *C. posadasii* has been named that is clinically identical to *C. immitis* exists as a mycelium in soil; humans are infected by inhalation of airborne arthroconidia from contaminated soil or dust. Sixty percent of infected immunocompetent individuals are either asymptomatic or have trivial upper respiratory tract symptoms. Of the remainder, most have self-limited lower respiratory tract disease.

*Coccidioides immitis* infection has a more aggressive course in the HIV-infected patient who lacks the specific cellular immune response which is necessary to control the fungus. A prospective study of patients living in an endemic area demonstrated a 25% incidence of active coccidioidomycosis over

3.5 years.<sup>85</sup> A CD4+ count  $<250/\text{mm}^3$  and a diagnosis of AIDS were identified as risk factors for the development of active coccidioidomycosis in this study.<sup>85</sup> The median CD4+ count among patients described in a large retrospective series was approximately  $100/\text{mm}^3$ .<sup>86</sup> Primary infection causes at least some of the active coccidioidomycosis that occurs among HIV-infected patients who reside in endemic areas, whereas reactivation of latent infection causes disease that occurs in patients who have not been in an endemic region in the past 6 months.<sup>87</sup>

Diffuse pneumonia is the most common presentation of coccidioidomycosis in the patient with AIDS.<sup>86-90</sup> Most patients have fever, chills, cough, weight loss, and a chest radiograph that shows diffuse reticulonodular infiltrates which can resemble PCP. Diffuse coccidioidal pneumonia is unusual in patients without immune dysfunction and HIV-infected patients who present with this form of disease have lower CD4+ counts (mean  $55/\text{mm}^3$ ) than those with focal pneumonia due to *C. immitis*.<sup>90</sup> Diffuse pulmonary coccidioidomycosis in the setting of HIV is associated with a mortality rate of approximately 70%.<sup>86,90</sup> Focal lung infection presents as acute community-acquired pneumonia in HIV-infected patients who are less immunocompromised.<sup>86</sup> Fever, cough with or without sputum production, and pleuritic chest pain are typical symptoms. Chest radiography shows a focal alveolar infiltrate or nodule; hilar lymphadenopathy and pleural effusions are also seen.

The frequency of extrathoracic coccidioidomycosis is greater among patients with HIV infection than among their HIV-uninfected counterparts.<sup>86</sup> The most common form of disseminated coccidioidomycosis in patients with HIV is meningitis which causes headache, lethargy, impaired mentation, and fever that typically develop over weeks to months. This presentation is similar to that which is seen among patients without HIV except concomitant diffuse pulmonary disease is more likely to be present in the patient with HIV.<sup>90</sup> Typically, the WBC count in the CSF is  $>50/\text{mm}^3$ , with a predominance of lymphocytes, a low glucose concentration, and a high total protein concentration.<sup>89</sup>

Other manifestations of coccidioidomycosis include cutaneous disease, osteoarticular disease, extrathoracic lymphadenopathy (especially inguinal), and hepatosplenic coccidioidomycosis which causes fever, inanition, and hepatosplenomegaly.<sup>86</sup> A form of the disease which appears unique to those with HIV causes fever, weight loss, and serologic positivity without evidence of involvement of any specific organ.<sup>87</sup> A small number of patients have persistent serologic positivity without evidence of active disease, a situation that is unusual among patients without HIV infection.<sup>91,92</sup> Most of these patients will develop active coccidioidomycosis as their CD4+ counts decrease.<sup>91</sup>

Direct visualization of *C. immitis* spherules in clinical specimens and histopathologic sections, growth of the fungus in culture, and antibody testing form the basis for the diagnosis of coccidioidomycosis. Unlike *Cryptococcus neoformans* and *H. capsulatum*, antigen detection methods are not available for *C. immitis*. Among patients with respiratory symptoms or chest radiographic abnormalities, examination and culture of expectorated sputum are useful. If sputum is unavailable, bronchoscopy with bronchoalveolar lavage should be performed. Cytologic methods using Papanicolaou and Grocott-Gomori methenamine silver nitrate stains provide the highest yield, and both stains will detect *Pneumocystis jiroveci* as well. Because

the sensitivity of cytologic staining for *Coccidioides* does not approach 100%, a negative stain does not exclude the diagnosis of coccidioidomycosis. In one report, staining or culture of respiratory specimens was diagnostic in about two-thirds of HIV-infected patients with pulmonary coccidioidomycosis.<sup>90</sup> In this study, histopathologic examination or culture of transbronchial biopsy specimens was diagnostic in all the remaining cases.<sup>90</sup> Similarly, direct examination and culture of biopsied tissue are usually diagnostic in patients with extrapulmonary, non-meningeal infection. Blood cultures are unreliable.<sup>87</sup> *C. immitis* is seen rarely in the CSF of patients with meningitis but five of 10 patients in one report had positive CSF cultures.<sup>86</sup>

Serologic testing is important in the diagnosis and management of coccidioidomycosis but antibody measurement is less reliable in the HIV-infected patient than in the immunocompetent patient.<sup>87</sup> Anticoccidioidal IgM antibody, which was originally detected with the tube precipitins (TP) method, transiently appears in the serum soon after acute infection. IgM antibody titers are not useful for monitoring response to therapy, nor are they useful for diagnosing meningitis. Anticoccidioidal IgG antibody, which was originally detected with complement fixation (CF), appears in the serum later. IgG titers reflect the activity of disease and serial measurements are used to monitor therapeutic response. The presence of anticoccidioidal IgG in the CSF strongly supports the diagnosis of meningitis and, as in serum, titers decrease with successful treatment. From 68% to 83% of HIV-infected patients with coccidioidomycosis have at least one positive serologic test at the time of diagnosis.<sup>86,90</sup> Most of those with negative serum antibody assays have diffuse pulmonary coccidioidomycosis.<sup>86,90</sup>

The site(s) and severity of disease are the key considerations in the treatment of coccidioidomycosis. Non-comparative clinical trials suggest that oral azoles are the drugs of choice for the management of chronic pulmonary, extrapulmonary non-meningeal, and meningeal coccidioidomycosis.<sup>93-97</sup> Itraconazole and fluconazole appear to be of roughly equal efficacy for the treatment of extrapulmonary non-meningeal disease.<sup>98</sup> However, fluconazole is generally preferred because it is better absorbed and is less likely to interact with other drugs commonly used in the management of patients with AIDS.<sup>87</sup>

Because the trials cited in the preceding paragraph included relatively few or no HIV-infected patients, the treatment recommendations for coccidioidomycosis in the setting of HIV are largely empiric. Treatment is indicated for all forms of coccidioidomycosis in patients with HIV.<sup>99</sup> Patients with non-meningeal disease who require hospitalization, including those with diffuse reticulonodular pneumonia, should receive intravenous amphotericin B 1 mg/kg/day as initial therapy. Once clinical improvement occurs, which may take several weeks and 500–1000 mg of amphotericin B, therapy is switched to oral fluconazole 400 mg/day or itraconazole 200 mg twice daily. Some experts favor initial combination therapy with amphotericin B and an azole for diffuse pneumonia.<sup>87</sup> Oral fluconazole 400 mg/day is appropriate initial therapy for HIV-infected patients with focal pneumonia and for those with mild to moderate extrapulmonary non-meningeal disease.<sup>38</sup> There are no data regarding the use of lipid formulations of amphotericin B for coccidioidomycosis.

In one study of fluconazole for coccidioidal meningitis, nine of 50 patients were infected with HIV.<sup>96</sup> Fluconazole 800 mg/day was associated with a clinical response in six of



these nine patients, and this treatment is generally accepted as the primary therapy of choice for HIV-infected patients with coccidioidal meningitis.<sup>96</sup> Intrathecal amphotericin B is indicated when fluconazole therapy fails.<sup>99</sup> However, this therapy is technically demanding and is associated with significant adverse effects, including chemical arachnoiditis and direct neurotoxicity. The combination of an azole and intravenous amphotericin B appears useful in the management of the patient who has meningitis and diffuse pneumonia.

Patients who are seropositive for CF (IgG) anticoccidioidal antibodies but who lack clinical manifestations of coccidioidomycosis are candidates for oral azole therapy. Some experts treat these individuals with oral fluconazole 400 mg/day, with the goal of preventing disease. An alternative approach is to follow these patients every few months and intervene with therapy only if disease develops or if IgG titers increase significantly.

HIV-infected patients being treated for coccidioidomycosis are clinically evaluated and have serologic testing repeated every 3–4 months. A rising IgG titer suggests relapse and prompts more in-depth evaluation. Options for treating recrudescing disease include using a higher azole dose or switching from an azole to amphotericin B. For patients who no longer have clinical evidence of coccidioidomycosis and who have achieved undetectable IgG titers, the azole dose can be reduced to a “maintenance” level. Generally, patients who received 400 mg/day of fluconazole initially are given 200 mg/day, and those who received 800 mg/day (i.e., for meningitis) are switched to 400 mg/day for life-long suppression. At the current time, there are insufficient data to recommend the discontinuation of suppressive therapy for the HIV patient whose CD4+ count increases in response to HAART.<sup>38</sup> Patients with meningitis receive life-long secondary prophylaxis with an azole regardless of their HIV status.<sup>99</sup>

## Penicilliosis

Infection with the dimorphic fungus *Penicillium marneffei* causes penicilliosis, an endemic mycosis found in Southeast Asia and southern China. The number of cases of penicilliosis abruptly increased in Thailand concomitant with the burgeoning AIDS epidemic in that country in the early 1990s.<sup>100</sup> Typically, patients with this opportunistic infection have CD4+ counts <50/mm<sup>3</sup> or previous or concomitant AIDS-defining conditions.<sup>101</sup> The most common manifestations of disseminated penicilliosis in the AIDS patient are fever, anemia, weight loss, and skin lesions.<sup>101</sup> The characteristic skin papules have a central umbilication and resemble the lesions of molluscum contagiosum and cryptococcosis.<sup>100,101</sup>

Visualization of septate, round and ovoid yeast forms on Wright-stained bone marrow aspirates and touch preparations from skin lesion and lymph node biopsies is sufficient for the presumptive diagnosis of disseminated penicilliosis.<sup>101</sup> Histopathologic sections of biopsy specimens stained with Grocott-Gomori methenamine silver nitrate or periodic acid-Schiff (PAS) contain similar forms.<sup>100</sup> Definitive diagnosis requires culture, and bone marrow and lymph node tissue culture appear to be most sensitive; the sensitivity of blood culture in one series was 76%.<sup>101</sup>

In an open-label clinical trial, 72 of 74 patients with HIV and disseminated penicilliosis responded to 2 weeks

of amphotericin B 0.6 mg/kg/day, followed by itraconazole 200 mg PO twice daily for 10 weeks.<sup>102</sup> In a subsequent study, itraconazole 200 mg/day was found to be highly effective for the prevention of relapse.<sup>103</sup> Patients with HIV and penicilliosis should receive treatment identical to the regimens used in these two studies.<sup>38</sup> Currently, there are insufficient data to recommend discontinuing secondary prophylaxis in the patient whose CD4+ count increases in response to HAART.<sup>38</sup>

## Other fungal diseases in the patient infected with HIV

Disseminated and central nervous system aspergillosis were included in the original case definition of AIDS but later were removed from the list of opportunistic diseases predictive of underlying cellular immune deficiency.<sup>3,104</sup> However, invasive aspergillosis emerged as a problem in the early 1990s among patients with advanced AIDS.<sup>105–108</sup> Classic risk factors for aspergillosis (e.g., neutropenia, steroid use) are often but not always present in these cases.<sup>108</sup> In one series, neutropenia (absolute neutrophil count <1000/mm<sup>3</sup>) was present in about 50% of HIV-infected patients with invasive aspergillosis.<sup>109</sup> Advanced HIV disease is probably an independent risk factor for invasive aspergillosis.<sup>109</sup>

*Aspergillus fumigatus* is the species most commonly recovered from HIV-infected patients with aspergillosis.<sup>109</sup> The lung and the central nervous system are the most frequent sites of disease.<sup>106,107,109</sup> Patients with invasive pulmonary aspergillosis typically have a subacute illness with cough, fever, dyspnea, chest pain, and, less commonly, hemoptysis.<sup>107,109</sup> Radiographic lung findings are variable but upper lobe cavitory disease is relatively common.<sup>105,108,109</sup> Ulcerative tracheobronchitis and obstructive bronchitis are other forms of pulmonary aspergillosis seen in the HIV-infected patient; fever, cough, dyspnea, and wheezing are common manifestations.<sup>107–110</sup> Bronchoscopic findings in patients with tracheobronchitis include ulcerative lesions and necrosis, sometimes with a pseudomembrane.<sup>110</sup>

If a patient has a compatible illness and radiographic abnormalities, the finding of *Aspergillus* in respiratory secretions is highly suggestive of pulmonary aspergillosis.<sup>109</sup> Definitive diagnosis requires demonstration of dichotomously branching, septated hyphae forms in lung tissue and growth of the fungus in culture.<sup>108</sup> Transthoracic needle aspiration of a pulmonary lesion appears to have a greater diagnostic yield than transbronchial biopsy in this setting.<sup>105</sup> Biopsy and culture are also necessary to confirm the diagnosis of invasive aspergillosis at sites other than the lung.

Treatment of invasive aspergillosis in the patient with HIV does not differ from the approach used in non-HIV infected patients. The reader is referred to Chapter 11 in this book.

Blastomycosis, a systemic illness caused by the dimorphic fungus *Blastomyces dermatitidis*, is endemic in the midwestern, south central, and some areas of the southeastern United States. Primary infection, which may be subclinical, occurs in the lung after inhalation of conidia. In contrast to histoplasmosis and coccidioidomycosis, blastomycosis seems to be infrequent among patients with HIV infection.<sup>111</sup> Two patterns of disease are associated with *B. dermatitidis* infection in the HIV population: localized pulmonary disease and disseminated

extrapulmonary disease.<sup>111</sup> Symptoms of localized pulmonary disease include fever, weight loss, cough, dyspnea, and chest pain. Chest radiography may show focal lobar consolidation, nodular opacities or a diffuse pattern of involvement. Disseminated blastomycosis often presents fulminantly with widespread multiorgan disease, including involvement of the skin and central nervous system. Compared to immunocompetent patients, those with AIDS and blastomycosis are more likely to have diffuse pulmonary infiltrates, respiratory failure, multiorgan disease, and fatal outcome.<sup>111</sup>

Immunocompromised patients with blastomycosis, including those with AIDS, should receive amphotericin B as initial therapy.<sup>112,113</sup> After a primary course of at least 1 g of amphotericin B, therapy may be switched to itraconazole 200–400 mg/day, to prevent relapse. Initial therapy with itraconazole may be considered for those with mild disease but this recommendation is based on data that support the use of itraconazole for HIV-uninfected patients with blastomycosis that is not life-threatening.<sup>114</sup> The appropriate duration of antifungal therapy for the HIV-infected patient with blastomycosis is unknown, and life-long suppressive therapy should be strongly considered.

Sporotrichosis is also infrequent among HIV-infected patients.<sup>115,116</sup> Conidia or hyphae of the dimorphic fungus *Sporothrix schenckii* are usually acquired through percutaneous inoculation of infected organic material such as sphagnum moss and decaying vegetation; less commonly, conidia are inhaled. In contrast to the localized lymphocutaneous disease seen in immunocompetent patients, disseminated disease, which may involve the central nervous system, is reported most often in patients with HIV infection.<sup>115–117</sup> The treatment of sporotrichosis is essentially the same as that described above for blastomycosis.<sup>118</sup>

Infection with the dimorphic fungus *Paracoccidioides brasiliensis* causes paracoccidioidomycosis, which is the most prevalent systemic mycosis in Latin America. For unexplained reasons, paracoccidioidomycosis has been reported only rarely among patients with HIV infection residing in endemic areas.<sup>119</sup> The most common presentation is a febrile wasting illness with lymphadenopathy, hepatomegaly, splenomegaly, and rash. Amphotericin B is recommended for initial therapy.

## Drug interactions associated with antifungal therapy in the patient infected with HIV

The most important drug interactions involve altered metabolism of a drug due to inhibition or induction of the cytochrome P450 enzyme system, in particular the 3A4 isoform (CYP3A4) which is located in hepatocytes and small intestinal cells. In addition, drugs can affect the function of P-glycoprotein, a cellular transport protein which resides in the small intestine.<sup>120</sup> The HIV-protease inhibitors and non-nucleoside reverse transcriptase inhibitors, which are substrates for cytochrome P450 enzymes, may act as inducers or inhibitors of enzymatic activity, depending upon on the particular combination under consideration.<sup>120</sup> Ritonavir, a protease inhibitor, is a potent inhibitor of both P450 and P-glycoprotein; efavirenz, a non-nucleoside reverse transcriptase inhibitor, can inhibit or induce P450 activity.<sup>120</sup> Co-administration of these drugs with azole

antifungals, which are also P450 substrates, is associated with clinically relevant interactions (Table 19-1). Ketoconazole and itraconazole are potent CYP3A4 inhibitors; fluconazole also inhibits CYP3A4 but only in high doses.<sup>120</sup> Voriconazole, which is metabolized primarily by isoform CYP2C9, inhibits metabolism of other drugs by a CYP3A4 or CYP2C9 pathway.<sup>123</sup> Careful consideration of drug interactions is required before co-administration of azole antifungals and antiretroviral drugs. Dose adjustments are often necessary and some combinations are contraindicated.<sup>121</sup>

Compared with the azoles, drug interactions are much less common with the echinocandins which are poor substrates for cytochrome P450 enzymes and which do not inhibit P-glycoprotein. However, serum levels of caspofungin are reduced in the presence of efavirenz; when these two drugs are co-administered, caspofungin should be given at a dose of 70 mg/day.<sup>124</sup> Drug interactions involving micafungin and anidulafungin have not been recognized to date.<sup>124</sup>

Itraconazole capsules, but not the suspension, require an acid environment for solubilization and absorption.<sup>125</sup> Therefore, efficacy may be diminished in patients with HIV-associated gastropathy, as well as in those with impaired gastric acid secretion due to prior gastric resection, old age, and the use of H<sub>2</sub>-receptor blockers, proton pump inhibitors or antacids.<sup>125</sup> The buffered formulation of the nucleoside reverse transcriptase inhibitor didanosine contains magnesium hydroxide, and co-administration of this drug impairs the absorption of itraconazole capsules. Gastric pH does not affect the absorption of voriconazole or fluconazole.

Overlapping drug toxicities and side effects may limit options for antifungal therapy in the patient with AIDS. The azoles, the protease inhibitors, and the reverse transcriptase inhibitors, in particular nevirapine, are all potentially hepatotoxic. 5FC is toxic to the rapidly dividing cells of the gastrointestinal tract and hematopoietic cells. Overlapping toxicity is likely when 5FC is co-administered with other drugs that suppress bone marrow, such as the nucleoside reverse transcriptase inhibitor zidovudine or trimethoprim-sulfamethoxazole, which most AIDS patients take for PCP prophylaxis. Many antiretroviral drugs can cause diarrhea, which concomitant administration of 5FC can worsen.

## Prevention of fungal infection in the patient infected with HIV

The ideal approach to prevention of fungal infections in the HIV-infected patient is to maintain or improve the integrity of the cellular immune system through the use of HAART. For a variety of reasons, not all HIV-infected patients who are candidates for therapy receive HAART, and among those that do, not all achieve optimal immunologic responses. With this in mind, the CDC periodically publishes recommendations for the prevention of HIV-related opportunistic infections.<sup>7</sup> These recommendations focus on prevention of exposure, primary prevention of disease (primary prophylaxis), and prevention of recurrent disease (secondary prophylaxis, “maintenance” or “suppressive” therapy). Prevention of recurrent disease is considered for each fungal infection in preceding sections of this chapter.

*Candida* species are common colonizers of mucocutaneous surfaces, and avoidance of exposure is not feasible. Although

**Table 19-1** Drug interactions involving selected azole antifungals and co-administered HIV protease inhibitors and HIV non-nucleoside reverse transcriptase inhibitors\*

Antiretroviral medication	Fluconazole	Itraconazole	Voriconazole
<b>Protease Inhibitors</b>			
Amprenavir and fosamprenavir		No data. Potential bidirectional interaction. Monitor for toxicity	No data. Potential bidirectional interaction. Monitor for toxicity
Atazanavir		No data. Potential bidirectional interaction. Monitor for toxicity	No data. Potential bidirectional interaction. Monitor for toxicity
Darunavir (given with ritonavir 100 mg bid)		No data. Use with caution. Itraconazole dose: $\leq 200$ mg/day	Ritonavir 100 mg bid decreases AUC of voriconazole by 39%. Co-administration is not recommended
Indinavir	No significant interaction	Decrease indinavir dose to 600 mg every 8 h. Itraconazole dose: $\leq 200$ mg/day	No significant interaction unless boosted with ritonavir
Lopinavir (co-formulated with ritonavir at dose of 100 mg bid)		Itraconazole levels increased. Use with caution. Itraconazole dose: $\leq 200$ mg/day	Ritonavir 100 mg bid decreases AUC of voriconazole 39%. Co-administration is not recommended
Nelfinavir		No data. Potential bidirectional interaction. Monitor for toxicity	No data. Potential bidirectional interaction. Monitor for toxicity
Ritonavir <sup>†</sup>	No significant interaction	No data. Potential bidirectional interaction. Monitor for toxicity. Dosage adjustment may be needed with itraconazole dose greater than 400 mg/day	Contraindicated with ritonavir >400 mg bid
Saquinavir (given with ritonavir 100 mg bid)	Saquinavir AUC increased 50%. Dose adjustment not established	Bidirectional interaction has been observed. Dose adjustment not established	Ritonavir 100 mg bid decreases AUC of voriconazole 39%. Co-administration is not recommended
Tipranavir (given with ritonavir 200 mg bid)	Tipranavir AUC increased 50%. Fluconazole dose: $\leq 200$ mg/day	No data. Use with caution. Itraconazole dose: $\leq 200$ mg/day	Ritonavir 100 mg bid decreases AUC of voriconazole 39%. Co-administration is not recommended
<b>Non-nucleoside Reverse Transcriptase Inhibitors</b>			
Delaviridine	No significant interaction		Potential bidirectional interaction. Monitor for toxicity

(Continued)



**Table 19-1** Drug interactions involving selected azole antifungals and co-administered HIV protease inhibitors and HIV non-nucleoside reverse transcriptase inhibitors\*—cont'd

Antiretroviral medication	Fluconazole	Itraconazole	Voriconazole
Efavirenz	No significant interaction	Co-administration associated with decreased itraconazole levels. Co-administration is not recommended	Co-administration associated with 44% increase in efavirenz levels and 77% decrease in voriconazole levels. Co-administration is contraindicated
Nevirapine	Co-administration associated with increased nevirapine levels and increased risk of hepatotoxicity. Monitor for nevirapine toxicity	Co-administration associated with decreased itraconazole levels. Monitor antifungal efficacy	Potential bidirectional interaction, including decreased voriconazole levels. Monitor for toxicity and antifungal efficacy

\*Data reproduced from Peiperl et al.<sup>121</sup> and DHSS panel on Antiretroviral Guidelines for Adults and Adolescents.<sup>122</sup> Empty cells indicate lack of information and low theoretical risk.

†The most common use of ritonavir is as a pharmacologic booster to decrease metabolism and increase levels of a second protease inhibitor. As indicated in the table, certain protease inhibitors should not be used without ritonavir boosting. Some, such as amprenavir, fosamprenavir, atazanavir, and indinavir, are usually boosted with ritonavir but may be given to treatment-naïve patients without ritonavir. When ritonavir is used as a single protease inhibitor, the dose is 600 mg bid.

AUC, area under the curve for plasma concentration.

exposure to pigeon feces is a putative risk factor for cryptococcal infection, there are no data proving that avian feces are the primary environmental source for *C. neoformans*.<sup>48</sup> In any case, it is reasonable to recommend that HIV-infected persons avoid sites that are heavily contaminated with pigeon excrement.<sup>48</sup> Although HIV-infected persons living in or visiting areas endemic for histoplasmosis cannot completely avoid exposure to *H. capsulatum*, those with CD4+ counts <200/mm<sup>3</sup> should be advised to avoid activities that could expose them to large inoculum, such as cleaning out chicken coops, exploring caves, disturbing soil beneath bird roosting sites, and working in or demolishing old buildings.<sup>7</sup> Likewise, it is prudent for the HIV-infected person living in or visiting areas endemic for coccidioidomycosis to avoid extensive exposure to disturbed soil, as might occur with excavation or during dust storms.<sup>7,126</sup>

The main consideration for prevention of disease is chemoprophylaxis with an antifungal drug. In a randomized comparative trial, fluconazole 200 mg/day was more effective than clotrimazole troches 100 mg five times per day in preventing cryptococcosis, OPC, and esophageal candidiasis, but use of fluconazole was not associated with reduced overall mortality.<sup>47</sup> Beneficial effects were greatest among the subgroup of patients with CD4+ counts of <50/mm<sup>3</sup>.<sup>47</sup> In another prospective trial, weekly fluconazole effectively prevented mucosal candidiasis among HIV-infected women.<sup>10</sup> In a randomized, placebo-controlled, double-blind study, itraconazole significantly reduced the incidence of histoplasmosis and cryptococcosis among patients with advanced HIV disease living in histoplasmosis-endemic areas but a survival benefit was not demonstrated.<sup>127</sup> Primary prophylaxis with itraconazole can be considered for the patient with a CD4+ count <100/mm<sup>3</sup> who is at great risk of exposure to *Histoplasma* because of occupation or residence in a hyperendemic area.<sup>7</sup> Otherwise, routine primary antifungal prophylaxis is not recommended.

## Conclusion

Depletion of CD4+ T lymphocytes predisposes HIV-infected individuals to a variety of opportunistic processes, including mycotic infections. Although advances in antiretroviral therapy have resulted in successful preservation and reconstitution of immune function in many patients infected with HIV, fungal pathogens remain a significant problem. Mucosal candidiasis is almost universal among patients with advanced AIDS. The other major opportunistic yeast, *C. neoformans*, is the most common fungus to cause life-threatening infection in this population. Histoplasmosis, coccidioidomycosis, and penicilliosis, the main endemic mycoses encountered among HIV-infected patients, are major causes of disease in their respective areas of endemicity. Amphotericin B remains the mainstay for initial therapy of most serious fungal infections in the AIDS population. Lipid formulations are preferred in some situations. Azoles play a major role in consolidation and suppressive therapy but drug interactions commonly occur when they are co-administered with HAART. The optimal intervention to prevent fungal infections in patients with HIV is to lower the risk through the use of HAART, a strategy which has been shown to be successful for patients who know their HIV status and who have access to healthcare and antiretroviral medications.

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# Invasive fungal infections in cancer patients

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## Introduction

Invasive fungal infections (IFIs) represent a major complication in patients with cancer, especially those with hematologic malignancies, and hematopoietic stem cell transplant (HSCT) recipients.<sup>1,2</sup> Highly intensive chemotherapeutic regimens and new antineoplastic drugs have contributed to an increase in the incidence of IFI<sup>3</sup> which continue to be associated with high morbidity and mortality.<sup>4</sup>

This chapter will highlight the important concepts guiding the management of IFI in cancer patients, including epidemiologic trends, new risk factors, and timetable of infections, pathogens and therapy and prevention of these infections. For more details on infections caused by specific fungi, please refer to the corresponding chapters. Infections caused by *Pneumocystis jiroveci* are discussed in Chapter 17 and will only be briefly mentioned.

## Epidemiology of invasive fungal infections in cancer patients

### Background

The epidemiology of IFI in patients with cancer varies according to the type of antineoplastic therapies deployed: myeloablative, requiring HSCT (usually for hematologic malignancies), or conventional non-myeloablative (predominantly in solid tumors). Patients with solid tumors are at increased risk of invasive candidiasis. Indeed, most candidemias in cancer patients reported in recent studies occurred in those with solid cancer, frequently following complicated surgery for treatment of the tumor.<sup>5-8</sup> The classic risk factors for invasive candidiasis are usually present, including candidal colonization of the gut as a result of exposure to multiple antibiotics, loss of the integrity of the gut mucosal barrier because of parenteral nutrition, gut disruptive surgery, chemotherapy and/or radiation therapy and systemic immunosuppression resulting from the latter two treatment modalities.

By contrast, patients with hematologic malignancies acquire invasive candidiasis more frequently during neutropenia, especially if they have mucositis or gut graft-versus-host disease

(GvHD).<sup>9-13</sup> Unlike patients with solid tumors in whom most infections are caused by *Candida albicans*, non-*albicans Candida* spp. account for a higher proportion of yeast infections in patients with hematologic cancer. In addition, these patients are particularly susceptible to invasive mould infections (IMIs), particularly aspergillosis<sup>14</sup> (Table 20-1).

### Increasing incidence of invasive fungal infections

A review of 8124 autopsies performed between 1978 and 1992 revealed a rise in IFI from 2.2% (1978–82) to 3.2% (1983–87) and to 5.1% in the latter years ( $P<0.001$ ). The highest infection rates were found in aplastic syndromes (68%), followed by acute myeloid leukemia (AML) (25%) and AIDS (19%).<sup>15</sup>

### The changing landscape of invasive fungal infections in cancer patients

#### Decreasing candidiasis

Since the 1990s, fluconazole has been widely used for antifungal prophylaxis and treatment and has resulted in a steady decrease in the incidence of candidemia, at least partly attributed to a reduction in *C. albicans* infections and a relative rise in infections by non-*albicans Candida* species.<sup>10,16</sup>

#### Relative increase in aspergillosis and other opportunistic mould infections

The decrease in candidiasis has been associated with a relative increase in aspergillosis and other IMIs.<sup>17</sup> While *Aspergillus* spp., particularly *A. fumigatus*, account for the largest proportion of IMIs, the last decade has witnessed the emergence of other IMIs including non-*fumigatus Aspergillus* spp., *Fusarium* spp. and the agents of zygomycosis (mucormycosis).<sup>18,19</sup>

#### The zygomycosis question

Because zygomycetes are resistant to voriconazole, an association between increasing voriconazole use and the relatively higher prevalence of zygomycosis was suggested. However,

**Table 20-1** Estimated incidence of invasive fungal infections among hematopoietic stem cell transplant recipients and relationship to antifungal prophylaxis

Stem cell transplantation	Aspergillosis		Candidiasis	
	Without mould prophylaxis*	With mould prophylaxis*	Without yeast prophylaxis†	With yeast prophylaxis†
Allogeneic	5–10%	<5%	15–20%	<1%
Autologous	2–3%	Unclear	~5%	<1%

\*Mould prophylaxis with itraconazole, voriconazole, posaconazole, echinocandin, amphotericin B formulation.  
†Yeast prophylaxis with fluconazole, itraconazole, voriconazole, posaconazole, echinocandin, amphotericin B formulation.  
Most invasive fungal infections occur within 60 days after stem cell transplantation, with a bimodal distribution for aspergillosis (an early peak at approximately 3 weeks post transplant, and later peak at approximately 3 months following transplant).

increasing zygomycosis has been reported before the availability of voriconazole<sup>20</sup> at the same institution where a strong association between prior voriconazole use and breakthrough zygomycosis was subsequently made.<sup>19</sup> Additional data from the same center suggest that the prevalence of zygomycosis increased from 0.9% (1989–93) to 4% (1994–98), only to decrease to 3% for the 1999–2003 period (which includes the period after which voriconazole became widely used at the same institution).<sup>21</sup>

These data suggest that factors other than voriconazole exposure may be playing a role in the emergence of zygomycosis. Previously reported variations in the prevalence of zygomycosis may be due to temporal fluctuation in their epidemiologic reservoir and may possibly explain the lower prevalence of zygomycosis during the latter period of the study. Improvements in supportive care, including the availability of newer antifungal agents (voriconazole, echinocandins), may be increasing the proportion of the immunocompromised population who are now surviving long enough to become colonized and infected by the zygomycetes. This is supported by the characteristics of patients with zygomycosis: severely immunosuppressed patients who are either suffering relapse of hematologic cancer or more commonly are recipients of unrelated, mismatched or haploidentical allogeneic HSCT with severe GvHD on intensive immunosuppressive therapies. Not uncommonly, these patients have concomitant infections with various opportunists including other fungi, because of severe immunosuppression.<sup>22–29</sup>

Zygomycosis is indeed extremely rare in other settings as shown by the very low prevalence of zygomycosis (four of 3316 patients; 0.12%) observed in all eight randomized prophylaxis trials in patients with hematologic cancers (including allogeneic HSCT) published between 2004 and 2007.<sup>30–37</sup>

A recent report in abstract form analyzed 77 cases of proven and probable zygomycosis among HSCT patients (October 2001–September 2006) at the 25 academic TRANSNET (Transplant-Associated Infection Surveillance Network) centers. The 1-year cumulative incidence was 3.8 cases per 1000 HSCT recipients with a median time from transplantation of 135 days. The incidence of zygomycosis increased from 0.04% to 0.21% during the 5-year course of the study.<sup>38</sup> Risk factors among the HSCT patients included receipt of corticosteroids

(77%), neutropenia (51%), and active diabetes (43%). Voriconazole was the most frequent antifungal agent given prior to infection (53%). An association between voriconazole prophylaxis and zygomycosis was suggested although the study was not designed to answer this question.

Taken together, the emergence of zygomycosis and its relationship to voriconazole exposure remain to be convincingly demonstrated.

## Newly identified sources

Patients with IMI may acquire their infection in the hospital or in the community from different sources, including air and water. Opportunistic moulds including *Aspergillus* and *Fusarium* spp. are present in the air, water and water-related surfaces of hospitals caring for cancer patients and molecular studies have confirmed the relatedness between patients' strains and water-related strains in aspergillosis and fusariosis. Hospital water should be considered a potential source of nosocomial IMIs and particular attention given to decreasing patient exposure during the periods of severe immunosuppression.<sup>39–41</sup> Additional potential sources of pathogenic fungi include probiotics (capsules containing *Saccharomyces cerevisiae* used for treatment and prevention of *Clostridium difficile*-associated diarrhea).<sup>42</sup>

## Newly reported risk factors

Several agents have been associated with increasing the risk for IFI.

- **Purine analogs.** Fludarabine, cladribine, deoxycorformycin used in treating hematologic cancers.<sup>43</sup>
- **Monoclonal antibodies.** Alemtuzumab (CAMPATH) is an anti-CD52 monoclonal antibody which produces profound T cell depletion and is used in lymphoreticular malignancies and for the purpose of T cell depletion in allogeneic HSCT recipients.<sup>44</sup> Two monoclonal antibodies (infliximab, a tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) antagonist, and daclizumab, an interleukin-2 (IL-2) receptor antagonist) are used for treating steroid-resistant GvHD in allogeneic HSCT recipients and have been shown to increase the risk of IMI in this setting.<sup>45–48</sup>



## Risk of invasive fungal infections in cancer patients

### Key principles

Cancer patients represent a heterogeneous group with respect to risk of IFI. The risk of IFI in cancer patients is the result of the interaction between host, pathogen, and environmental exposure. Invasive fungal infections occur when an imbalance develops between the weakened protective defense mechanisms of the host and the virulence factors of the offending pathogen.

- Host.

Net state of immunosuppression (type, degree and pace, and duration)

Immunogenetics

Pharmacogenetics

Presence of tissue damage and organ dysfunction

- Pathogen: the virulence factors of various fungi that cause IFI in cancer patients.
- Environment: patient's degree of exposure to pathogens. This risk for infection is constantly evolving, depending on the continuous interactions between the host and epidemiologic exposure, increasing substantially during the course of therapy when many of these risk factors may be simultaneously present, only to decrease or even disappear when most risk factors resolve. As a result, the period of risk for infection is variable.

An appreciation of these risk factors is critical for the management of cancer patients. Constant monitoring of immune function is critical to determining the net state of immunosuppression and can be accomplished by a variety of tests such as neutrophil and lymphocyte counts and the drug levels of calcineurin inhibitors and other immunosuppressants.

Tables 20-2 and 20-3 provide a framework for determining the risk of IFI in cancer patients and stem cell transplant recipients.

### The host

#### Net State of Immunosuppression

The net state of immunosuppression represents the cumulative effect of the different types of immunosuppression caused by the underlying disease, prior and current therapies, co-morbidities, infections with immunomodulating viruses (cytomegalovirus (CMV), human immunodeficiency virus, others) iron overload and, in allogeneic HSCT recipients, the presence of severe GVHD and its therapy. These factors impair different host defenses, predisposing to different fungal pathogens (see Tables 20-2, 20-3).<sup>12,14,49-51</sup> Achievement of remission of the underlying malignancy is usually critical in reducing the net state of immunosuppression.

A role of the patient's immunogenetics and the pharmacogenomics of antineoplastic drugs is being increasingly recognized. Indeed, relationships between genetic variation and drug effect (pharmacogenomics) have been observed for a growing number of commonly used antineoplastic drugs. These genetic variations relate to the amount of exposure

to the specific agent and hence the patient's risk for various degrees of immunosuppression. An example is thiopurine methyltransferase (TPMT), an enzyme responsible for the degradation of azathioprine and mercaptopurine, both commonly used to treat acute leukemia. Patients with complete TPMT deficiency are at a nearly 100% risk of severe and potentially fatal hematologic toxicity, whereas heterozygotes are at intermediate risk (35%).<sup>52,53</sup>

Because of significant prolongation of severe myelosuppression, patients with these genetic variations are likely to be at a much higher risk for myelosuppression-related IFIs. Genetic variations with other antineoplastic agents are currently undergoing extensive investigation.

Similarly, the role of host response to opportunistic fungi has been explored in relatively small studies using a candidate gene approach. Single nucleotide polymorphisms (SNPs) of the following genes were identified as potential risk variables for the development of invasive pulmonary aspergillosis (IPA): Toll-like receptor (TLR) 1, IL-10 promoter, IL-10 1082\*G allele and G/G genotype, IL-15 +13689\*A allele and A/A genotype, transforming growth factor (TGF)- $\beta$  + 869 and T/T genotype, TNF- $\alpha$  -308\*A/A and variable numbers of tandem repeats at position -322 in the promoter region of the TNR2 gene, mannose-binding lectin (MBL), MBL-associated protein (MASP-2) and lung surfactant proteins SPA-2. An association was also suggested between TLR4 Asp299Gly/Thr399Ile polymorphisms and increased susceptibility to *Candida* bloodstream infections and between polymorphisms of the low-affinity Fc $\gamma$  receptors, FCGR2A, 3A and 3B genes and the risk for cryptococcosis.<sup>54-61</sup>

These exploratory studies give us a glimpse of what to expect from whole-genome studies which are likely to provide much better insights into the risk factors for IFIs. These studies, unfortunately, remain hugely expensive and require much larger sample sizes.

#### Tissue damage and metabolic and organ dysfunction

The risk of IFI increases in the presence of severe damage to the gut mucosal tissues (upper or lower alimentary tract mucositis following chemotherapy, radiation therapy or GVHD),<sup>2,62-64</sup> hyperglycemia, and renal, hepatic or respiratory failure.<sup>65-72</sup> Iron overload may also contribute to dysfunction of various organs.<sup>73</sup>

### Environmental exposure

#### Endogenous colonization

Patients undergoing antineoplastic therapy may become colonized with nosocomial pathogens, such as fluconazole-resistant *Candida* or *Aspergillus* species. After chemotherapy, these pathogens may cause an IFI as shown by the strong association between colonization and subsequent IFI.<sup>74,75</sup> Gut mucosal colonization is a requirement for invasive candidiasis which usually develops following translocation of *Candida* from the gut into the bloodstream.<sup>76</sup> Any situation which leads to breakdown in the intestinal mucosa, such as chemotherapy or radiotherapy-induced mucositis, or gut-disruptive surgery, increases the risk of invasive candidiasis. Disruption of skin integrity (e.g., indwelling catheters) may occasionally play a role in predisposing patients to these infections.<sup>77,78</sup>

**Table 20-2** Risk factors for invasive fungal infections in cancer patients

Etiology	Type of defect	Fungal infection
IMMUNOSUPPRESSION		
Underlying disease		
Aplastic anemia	Neutropenia♦	Invasive candidiasis, mould infections*
Acute leukemia	Neutropenia♦, T cell immunodeficiency♠	Invasive candidiasis, mould infections*
Lymphoma	T cell immunodeficiency♠	Mucosal candidiasis, cryptococcosis**
Treatment		
Myelosuppressive chemotherapy	Neutropenia♦	Invasive candidiasis, mould infections*
	T cell immunodeficiency♠	
Immunosuppressive agents		
Corticosteroid†	T cell immunodeficiency♠	Invasive candidiasis, mould infections*, cryptococcosis**
Purine analogs ††	T cell immunodeficiency♠	Mucosal candidiasis, hematogenous candidiasis
Monoclonal antibodies		
Alemtuzumab	T cell immunodeficiency♠	Mucosal and invasive candidiasis, mould infections*
GvHD		
Severe GvHD and its therapy	T cell immunodeficiency♠	Mould infections*
Immunomodulatory infections (e.g., CMV)	T cell immunodeficiency♠	Mucosal candidiasis, mould infections*
ORGAN DYSFUNCTION		
Malnutrition	T cell immunodeficiency♠	Mucosal candidiasis
Renal failure	Phagocytic dysfunction; T cell immunodeficiency♠	Mucosal candidiasis, invasive aspergillosis
Liver failure/cirrhosis	Phagocytic dysfunction; T and B cell immunodeficiency♠♣	Invasive candidiasis, cryptococcosis, coccidioidomycosis
Gut disruption of the integrity of the gut mucosa	Alimentary tract mucositis after chemotherapy or radiation therapy	Invasive candidiasis
	Gut GvHD	
	Gut-disruptive surgery	
	Total parenteral nutrition	
Skin breakdown	Venous catheters	Invasive candidiasis
	Extravasation of antineoplastic agents	
	Skin GvHD	
	Fungating lesions (mycosis fungoides)	

**Table 20-2** Risk factors for invasive fungal infections in cancer patients—cont'd

<b>Etiology</b>	<b>Type of defect</b>	<b>Fungal infection</b>
<b>MICROBIAL EXPOSURE</b>		
<b>Selection pressure</b>	Broad-spectrum antibacterial antibiotics	Invasive candidiasis
	Antifungal azoles	Invasive candidiasis ( <i>C. krusei</i> , <i>C. glabrata</i> )
	Antifungal echinocandins	Trichosporosis ( <i>T. asahii</i> )
<b>Epidemiologic exposure</b>		
Hospital	<i>Candida</i> spp.	Transmission on hands of healthcare workers, hospital equipment and contaminated solutions ( <i>Candida parapsilosis</i> )
	<i>Aspergillus</i> and other moulds	Waterborne and airborne sources ( <i>Aspergillus</i> spp., <i>Fusarium</i> spp., other moulds), contaminated IV solutions
Home	Opportunistic moulds ( <i>Aspergillus</i> , other)	Moist environment (air conditioning units, humidifiers)
Occupational, recreational	<i>Histoplasma capsulatum</i> , <i>Blastomyces dermatitidis</i> , <i>Cryptococcus neoformans</i>	Dog owners, pigeon fanciers, farmers, and individuals performing excavations
	<i>Aspergillus</i> spp.	Users of recreational marijuana
	<i>Sporothrix schenckii</i>	Gardeners, florists, mineworkers, and carpenters
Geographic	<i>Paracoccidioides brasiliensis</i>	Central and South America
	<i>H. capsulatum</i> , <i>B. dermatitidis</i>	Southeast, mid-Atlantic and central USA
	<i>Coccidioides immitis</i>	Southwest and Midwest USA, Central and South America
	<i>Penicillium marneffeii</i>	Southeast Asia
GvHD, graft versus host disease; CMV, cytomegalovirus. ♦Neutropenia: absolute neutrophil counts <500/μl; severe neutropenia: <100/μl for >10–14 days. ♣T cell immunodeficiency: absolute lymphocyte counts <700/μl, CD4+ <200/μl. ♣B cell immunodeficiency: absolute CD19+ B lymphocytes <100/μl. †Corticosteroids: prednisone dose equivalent of 20 mg/day × 30 days or 700 mg cumulative dose over 30 days. ††Purine analogs: fludarabine, cladribine, deoxycorformycin. *Mould infections: invasive aspergillosis, zygomycosis, fusariosis, scedosporiosis, others. **Associated with chronic immunosuppression.		

### Exposure to Opportunistic Fungi

Environmental exposure to *Aspergillus* spp. through contaminated heating/cooling systems or hospital water systems, *Candida* spp. from healthcare workers, and *Fusarium* spp. from hospital water systems have been associated with both sporadic cases and outbreaks of IFI among cancer patients.<sup>39-41,79,80</sup> Whether these infections are nosocomial or community acquired may be very difficult to determine in an individual patient.

Some community-acquired fungal infections are particularly common in certain geographic areas or with certain occupational exposures (see Table 20-2). Following chemotherapy, these pathogens may cause primary infection or reactivation of latent infection in a previously exposed host.

The risk factors for IFI in high-risk cancer patients, i.e., acute leukemia, and HSCT patients are shown in Table 20-4.

### Timetable of fungal infections in cancer patients (Table 20-5)

The posttransplant timetable of IFI in allogeneic HSCT recipients may be divided into three periods that correspond broadly to the pattern of immune deficiency and organ damage following therapy: preengraftment, immediate postengraftment, and late postengraftment. In contrast, the timetable for IFI for patients receiving gut-damaging cytotoxic chemotherapy, such as for acute leukemia, is mostly limited to the preengraftment phase.



**Table 20-3** Risk factors for invasive fungal infection after stem cell transplantation

Risk factors and risk category	Engraftment		Comments
	Pre	Post	
NET STATE IMMUNOSUPPRESSION			
UNDERLYING DISEASE : AML, AA, MDS, MM, non-first remission	+	++	
EXTENSIVE PRIOR THERAPY	+	++	
AGE > 40 YEARS	+	+	Children have lowest risk
SEVERE NEUTROPENIA † Risk † with severe AT mucositis	++		MAJOR PREENGRAFTMENT RISK
GRAFT REJECTION, FAILURE	++	++	
CD4 cytopenia <200 cells/μl		++	
IMMUNOMODULATING VIRUSES (CMV, other▲)		+?	Role in increasing risk unclear
RESPIRATORY VIRAL INFECTIONS: RSV, influenza, Para-3, other	+	+	
CONDITIONING REGIMEN			
Myeloablative	+	+	
Non-myeloablative (no significant neutropenia or AT mucositis)		+	Risk increases during GvHD\$
GRAFT CHARACTERISTICS			
HLA relatedness, allogeneic			
Matched unrelated, mismatched related, haploidentical	+	+	Matched-related has lowest risk
Graft manipulation: T cell depletion, CD34 selection		+	Slower immune reconstitution
SOURCE			
Stem cell		+	Risk lowest with marrow (? Less GvHD)
Cord (prolonged neutropenia, even with double cord)	++		
Dose of progenitor cells infused	+	+	
Autologous: <2 × 10 <sup>6</sup> /kg CD34+ cells/kg	+		longer neutropenia
Allogeneic	+	+	
GRAFT VERSUS HOST DISEASE (GvHD)		++	MAJOR POSTENGRAFTMENT RISK
Prophylaxis: steroid-containing	+	+	

**Table 20-3** Risk for invasive fungal infection after stem cell transplantation—cont'd

Risk factors and risk category	Engraftment		Comments
	Pre	Post	
Severity: ♦ Acute, severe, (grades III–IV) or clinically extensive, chronic		++	
Treatment: ♦ High-dose corticosteroids, monoclonal antibodies		++	
<b>GENETIC PREDISPOSITION</b>			
INFECTION	+?	+?	
GvHD	+?	+?	
<b>ORGAN DYSFUNCTION</b>			
GUT			
Alimentary tract mucositis after conditioning regimen	++		
Gut GvHD		++	
Total parenteral nutrition	?	?	
SKIN			
Central venous catheters	?	?	
Extensive, skin GvHD		?	
OTHER: Renal failure, liver or lung dysfunction	+	+	
<b>MICROBIAL EXPOSURE</b>			
ENDOGENOUS: reactivation of latent infection, colonization	+	+	
EXOGENOUS			Risk ↑ after engraftment in outpatient setting
Air, water, food, inanimate objects, healthcare worker, donor infection**	+	+	
Occupational, recreational, geographic and other	+	+	
<p>+, risk factor; ++, major risk factor;  +?, risk present but magnitude of risk is unclear  AML, acute myelogenous leukemia; AA, aplastic anemia; AT mucositis, alimentary tract mucositis; GvHD, graft versus host disease; MDS, myelodysplastic syndrome; MM, multiple myeloma; CMV, cytomegalovirus;  § GvHD (acute and chronic) and its therapy (particularly with higher dose corticosteroids) are major contributors to risk of infection.  † Severe neutropenia: &lt;100/μl for &gt;10–14 days. Excepted days of neutropenia according to stem cell source: 10–20 (peripheral stem cells); 20–30 (bone marrow and umbilical cord blood).  ▲ Other: human herpes virus 6, 7. Because these viral infections are usually associated with corticosteroid therapy, it is unclear whether they truly contribute to increasing the risk for other infections or whether they merely serve as a marker for severe immunosuppression.  ♦ High-dose corticosteroids: induction: 250–1000 mg methylprednisolone/day for 5 days vs lower dose &lt;200 mg methylprednisolone/day for 5 days. Maintenance: prednisone 1 mg/kg/day  ** Donor infection: CMV, other.</p> <p>Many risk factors for infection are closely related: for example, duration of neutropenia is related to host and transplant variables including underlying disease, extensive prior therapy, stem cell source (cord) and dose (low), conditioning regimen (myeloablative), graft failure or rejection, and others. Similarly, many risk factors that increase the risk for GvHD include stem cell source (peripheral stem cell higher than marrow), HLA relatedness (mismatched or unrelated donor higher risk than matched related), etc.</p>			

**Table 20-4** Risk Factors for invasive fungal infections in patients with acute leukemia and those undergoing allogeneic hematopoietic stem cell transplantation

Infection	Acute leukemia	Allogeneic stem cell transplant
Hematogenous candidiasis	Fungal colonization: antibacterial antibiotics Impaired mucosal barrier: (chemotherapy-induced alimentary tract mucositis, parenteral nutrition) Immunosuppression: • Neutropenia, prolonged and severe (< 100/ $\mu$ l for >14 days) • Therapy with high-dose corticosteroids • Non-achievement of complete remission	<ul style="list-style-type: none"> <li>• Similar to acute leukemia.</li> <li>• Risk continues after engraftment, particularly if severe gut GvHD present</li> <li>• Bacteremia</li> <li>• Cytomegalovirus disease?</li> </ul>
Aspergillosis, fusariosis	Fungal colonization and prior invasive mould infection Immunosuppression (same as above)	<ul style="list-style-type: none"> <li>• Similar to acute leukemia</li> <li>• Additional risk factors shown in Table 20-3</li> </ul>
Zygomycosis	Same as aspergillosis plus diabetic ketoacidosis, iron overload	Same as aspergillosis plus diabetic ketoacidosis, iron overload

Severe GvHD: acute, grades III-IV; clinically extensive, chronic.  
 High-dose corticosteroids: induction: 250–1000 mg methylprednisolone/day for 5 days vs lower dose < 200 mg methylprednisolone/day for 5 days.  
 Maintenance: prednisone 1 mg/kg/day.

## Preengraftment

The most common pathogens during this period are *Candida albicans* and *C. tropicalis*, in the absence of fluconazole prophylaxis, and *C. glabrata* and *C. krusei* when fluconazole prophylaxis is used.<sup>10,81-83</sup> Among autologous HSCT recipients, the rate of IFI is higher among patients with hematologic malignancies,<sup>84,85</sup> and is very low among patients with solid tumors.<sup>86</sup>

In addition, transplants associated with neutropenia lasting >2 weeks have a high risk for IMIs, including *Aspergillus* spp. (most commonly), *Fusarium* spp., zygomycetes and others.<sup>87,88</sup> Patients receiving allogeneic HSCT with stem cells obtained from bone marrow and from cord blood are typically those with late engraftment. By contrast, transplants with stem cells from the peripheral blood and transplants with reduced-intensity conditioning regimens have neutropenia of short duration, and therefore are at low risk of IFI in the early posttransplant period.<sup>89</sup> In autologous HSCT, IMIs occur very occasionally.<sup>90</sup>

## Immediate postengraftment

The risk factors for IFI during this period are mucositis, cellular immune dysfunction, and immunomodulating viruses. For allogeneic HSCT recipients, additional risk factors include severe acute GvHD and its therapy (see Tables 20-2 to 20-5). During this period, patients are at risk for IMIs (especially aspergillosis).<sup>90,91</sup> Since the widespread use of azole prophylaxis, chronic disseminated candidiasis is now rarely seen.<sup>92</sup>

## Late postengraftment

Aspergillosis and other IMIs continue to occur during this period and may develop sometimes very late (>1 year) after allogeneic HSCT.<sup>87,88,93</sup> The main risk factor for IMIs in this

period is severe T cell-mediated immunodeficiency (CMI) caused by chronic GvHD and its treatment. These patients usually are not neutropenic, but their neutrophils do not migrate to the site of infection.<sup>94</sup>

## Fungal infections of particular importance in cancer patients

### A practical clinicopathologic classification

Although most fungi responsible for IFI in cancer patients may cause disease in one or more organs, some fungi are more commonly associated with manifestations related to involvement of certain organ systems. Common presentations fall into three categories (Table 20-6).

1. Disseminated infection with fungemia, metastatic skin lesions and multiorgan involvement, usually caused by yeasts (*Candida* and *Trichosporon* spp. and *Blastoschizomyces capitatus*).<sup>95,96</sup> The clinical manifestations are dominated by the widespread hematogenous dissemination of yeasts to various sites with cytokine storm and multiorgan damage.
2. Localized sinopulmonary disease due to angioinvasive moulds (*Aspergillus* spp., zygomycetes, others). The clinical and radiologic manifestations are determined by the airborne route of inoculation and the angioinvasive nature of these infections, with resulting infarction and hemorrhage.
3. Mixed sinopulmonary and hematogenously disseminated presentation which shares the features of the above two forms; as with angioinvasive IMIs, these infections may be airborne with sinopulmonary presentation and with



**Table 20-5** Timetable and clinical syndromes of invasive fungal infections in cancer patients and in HSCT recipients

Infection and Time of Occurrence	Setting, Risk Factors	Clinical Manifestations
<b>DURING MARROW APLASIA*</b>		
Acute disseminated candidiasis	Severe neutropenia●, mucositis, no azole prophylaxis	Fever +/- hypotension, myalgias, skin lesions
Mould pneumonia †	Severe neutropenia●	Fever +/- dry cough, pleuritic chest pain
Mould sinusitis †		Fever + nasal discharge, headache, epistaxis
Invasive fusariosis	Severe neutropenia● +/- skin breakdown +/- onychomycosis	Fever + nodular skin lesions, with ecthyma gangrenosum-like appearance, target lesions, etc.
<b>AFTER MARROW RECOVERY OR ENGRAFTMENT (HSCT)*</b>		
<b>Early postengraftment 30–100 days</b>		
Chronic disseminated candidiasis	Prior severe neutropenia●, mucositis, no azole prophylaxis.	Fever, ↑ alkaline phosphatase, right upper quadrant pain, hepatosplenomegaly
<b>Early postengraftment 30–100 days and late (&gt;100 days) (HSCT)</b>		
Mould pneumonia†	Severe GvHD▲, high-dose corticosteroids▲ Among allogeneic HSCT recipients, cellular immune dysfunction, infection with immunomodulating viruses	Cough, fever
Mould sinusitis †	Same as above	nasal discharge, headache
<i>Pneumocystis</i> pneumonia	Same as above	Dry cough, fever, hypoxemia
<b>Any period</b>		
Catheter-related fungal infection	Venous catheter	Fever +/- rigors after manipulation of the catheter
GvHD, graft versus host disease *During aplasia: may occasionally occur after marrow recovery. ▲Severe GvHD: acute, grades III–IV; clinically extensive, chronic. ●Severe neutropenia: <100 Neutrophils/μl for >10–14 days. ▲High-dose corticosteroids: induction: 250–1000 mg methylprednisolone/day for 5 days vs lower dose <200 mg methylprednisolone/day for 5 days. Maintenance: prednisone 1 mg/kg/day. †Caused by <i>Aspergillus</i> spp. in the majority of cases. Other moulds include <i>Fusarium</i> spp., zygomycetes and others.		

infarction and hemorrhage and the resulting clinical and radiologic manifestations. Like yeast infections, hematogenous dissemination to various sites is present in the setting of a cytokine storm and multiorgan damage. This mixed presentation occurs with some moulds which are also capable of adventitious in vivo sporulation, in which yeast-like spores, or aleurioconidia, are formed in tissue and blood with resultant hematogenous dissemination, positive blood cultures, and multiple cutaneous lesions (hence the yeast-like clinical manifestations). Aleurioconidia facilitate the diagnosis because they are more likely to be detected in blood cultures and on microscopic

examination of tissue, fine needle aspirates or bronchoscopy specimens. The fungal genus that best exemplifies this pattern of involvement is *Fusarium*; other moulds associated with this presentation include *Scedosporium* spp., *Acremonium strictum*, *Paecilomyces* spp. and *Aspergillus terreus*.

## Specific fungal infections

Please refer to specific chapters for additional information on the pathogenesis of disease, clinical presentation, and diagnosis and management of specific infections.

**Table 20-6** Common opportunistic mycoses in cancer patients by organ system affected: a practical clinicopathologic classification

	Pathogen	Pathophysiology	Fungal form in tissue	Typical presentation		
				Sinusitis pneumonia	Blood cultures	Skin lesions
Yeasts	<i>Candida</i> spp.	Cytokemia	Yeast, hyphae	–	+	+
	<i>Trichosporon</i>	Cytokemia	Yeast, hyphae	–	+	+
	<i>Blastoschizomyces</i>	Cytokemia	Yeast, hyphae	–	+	+
Moulds	<i>Aspergillus</i> spp. †	Angioinvasive	Hyphae	+	–	–
	<i>Zygomycetes</i>	Angioinvasive	Hyphae	+	–	–
	Others●	Angioinvasive	Hyphae	+	–	–
Moulds with in vivo adventitious sporulation♦	<i>Fusarium</i> spp.	Angioinvasive+ cytokemia	Yeast, hyphae	+	+	+
	<i>S. prolificans</i>	Angioinvasive + cytokemia	Yeast, hyphae	+	+	+
	<i>Acremonium</i> spp.	Angioinvasive + cytokemia	Yeast, hyphae	+	+	+
	<i>Paecilomyces</i> spp.	Angioinvasive + cytokemia	Yeast, hyphae	+	+	+

*S. prolificans* = *Scedosporium prolificans*.  
 ●Includes agents of hyalohyphomycosis and phaeohyphomycosis (see text).  
 ♦Moulds with in vivo adventitious sporulation: these angioinvasive pathogens are capable of adventitious sporulation in which yeast-like conidia, or aleurioconidia, are formed in tissue with resultant hematogenous dissemination, positive blood cultures, and multiple cutaneous lesions. Aleurioconidia are more likely to be detected in blood cultures and on microscopic examination of tissue, fine needle aspirates or bronchoscopy specimens, hence facilitating the diagnosis.  
 †*Aspergillus terreus* is also capable of vivo adventitious sporulation and fungemia.

### Candidiasis (Table 20-7)

*Candida* species cause both mucosal infections and invasive candidiasis such as candidemia, acute and chronic disseminated candidiasis.<sup>97,98</sup> While *C. albicans* and *C. tropicalis* used to be the species most commonly isolated from blood and deep tissue sites in cancer patients,<sup>99,100</sup> the proportion of infections attributed to other species, such as *C. krusei*, *C. glabrata* and *C. parapsilosis*, is rising.<sup>10,101,102</sup> In the case of *C. glabrata* and *C. krusei*, this shift may be a result of the widespread use of fluconazole prophylaxis.

Invasive candidiasis presents most frequently as candidemia, with fever as the sole clinical manifestation. In neutropenic patients, invasive candidiasis may present with refractory fever, clinical deterioration, myalgias, and multiple disseminated nodular or papular erythematous lesions, which on biopsy and culture frequently yield *Candida tropicalis*.<sup>101</sup>

Although less common since the introduction of fluconazole prophylaxis, chronic disseminated candidiasis deserves particular attention because of its unique manifestations. The syndrome follows a period of prolonged neutropenia, usually in a patient with acute leukemia or HSCT recipients who have not received triazole prophylaxis. The clinical presentation includes prolonged irregular fever unresponsive to various antibiotics, nausea, vomiting, anorexia, and abdominal discomfort. Right upper quadrant tenderness may be present with or without hepatosplenomegaly. Elevation of serum alkaline

phosphatase levels is almost always present. Radiographic diagnosis is best obtained after recovery from neutropenia and consists of an abdominal ultrasound, computed tomography (CT) scan or magnetic resonance imaging (MRI) which will demonstrate a unique pattern of multiple abscesses in the liver and/or spleen or kidneys, known as “target lesions.” These radiographic findings are discussed in detail in Chapter 6.

### Aspergillosis

Invasive aspergillosis is now the leading cause of IFI in patients with hematologic cancer and probably the most common cause of infection-related death. As mentioned before, aspergillosis affects almost exclusively patients with acute leukemia during neutropenia and allogeneic HSCT recipients. In the latter population, the infection may occur early post transplant (during neutropenia) or after engraftment, in patients with severe impairment of CMI.<sup>103</sup> Any factors that result in prolonged neutropenia and/or worsening CMI (such as severe GvHD and use of high doses of corticosteroids<sup>93,101</sup>; see Tables 20-2 to 20-5) increase the risk for aspergillosis. The emergence of aspergillosis among myeloma patients who have undergone allogeneic HSCT can be viewed in the context of cumulative CMI deficits as a result of the chronic administration of corticosteroids used for therapy of myeloma.<sup>104,105</sup>

Because aspergillosis is usually acquired through inhalation of airborne aspergilli, the most common disease manifestations

**Table 20-7** Findings suggestive of an invasive fungal infection in cancer patients

Infection	During aplasia	After recovery
ALL	Fever persisting or new, in a patient at risk for fungal infection and known to be colonized by a fungus	
YEASTS		
	<b>Skin rash:</b> Nodular: painless subcutaneous nodules ( <i>Candida</i> spp, others) Maculo-papular: <i>Candida</i> spp, rarely <i>Trichosporon</i> spp., <i>Malassezia furfur</i> , <i>Cryptococcus neoformans</i> , Pustular: <i>Candida</i> spp, rarely <i>Malassezia furfur</i> Ulcerative, necrotic: <i>Trichosporon</i> spp., <i>Candida</i> spp Purpura fulminans-like: <i>Candida</i> spp.	Same as during aplasia, PLUS: Upper abdominal pain, ↑ serum AP Positive findings on abdominal imaging ♠
	Myalgia, muscular tenderness, ↑ serum CPK (esp. <i>C. tropicalis</i> )	Chorioretinal lesions Joint/bone pain/unexplained Acute renal failure, unexplained
Laboratory	Blood cultures, ↑ serum β-d-glucan levels	↑ serum β-d-glucan levels
Abdominal imaging ♠		♠ Multiple small lesions in liver, spleen, kidneys
MOULDS AND DIMORPHIC-LIKE FUNGI §		
	Dry cough, pleuritic chest pain, pleural rub	Same as during aplasia PLUS
	Facial pain, nasal discharge, hard palate ulceration	Chorioretinal lesions ( <i>Fusarium</i> spp) Joint/bone pain unexplained
	<b>Skin rash</b> Maculopapular: <i>Fusarium</i> spp. Pustular: <i>Fusarium</i> spp. Ulcerative, necrotic: <i>Aspergillus</i> , <i>Fusarium</i> , hyalohyphomycetes, phaeohyphomycetes, zygomycetes Target lesion (rare): <i>Fusarium</i> spp. Extremity cellulitis at site of skin breakdown: <i>Fusarium</i> spp., rarely <i>Aspergillus</i> spp., others Myalgia, muscular tenderness, ↑ serum CPK: <i>Fusarium</i> spp.	
Laboratory	↑ Serum <i>Aspergillus</i> antigen†, β-d-glucan levels Blood cultures: <i>Fusarium</i> spp., <i>Scedosporium</i> spp, others	↑ Serum <i>Aspergillus</i> antigen♦, ↑ β-d-glucan levels
CT scan*	Nodular, halo sign or wedge-shaped infiltrates Abnormal sinus CT scan	Nodular, air crescent sign, cavitation Abnormal sinus CT scan
DIMORPHIC FUNGI **		
	Skin rash Papular: <i>P. marneffei</i> Maculopapular: <i>H. capsulatum</i> , <i>C. immitis</i> , <i>P. marneffei</i> Pustular: <i>B. dermatitidis</i> , <i>P. marneffei</i> Erythematous: <i>C. immitis</i>	Same as during aplasia

(Continued)



**Table 20-7** Findings suggestive of an invasive fungal infection in cancer patients—cont'd

Abdominal imaging, CPK, creatine phosphokinase; AP, alkaline phosphatase.

♦ Serum *Aspergillus* galactomannan titers.

\*CT scan: computed tomography scan.

♣ultrasound, CT scan, magnetic resonance imaging.

§Dimorphic-like fungi include moulds with in vivo adventitious sporulation: these angioinvasive pathogens are capable of adventitious sporulation in which yeast-like conidia, or aleurioconidia, are formed in tissue and blood with resultant hematogenous dissemination, positive blood cultures, and multiple cutaneous lesions. Pathogens include *Fusarium* spp., *S. prolificans*, *Acremonium* spp., *Paecilomyces* spp. and *Aspergillus terreus*.

\*\*Dimorphic (endemic) mycoses: *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis*, *Paracoccidioides brasiliensis* and *Penicillium marneffei*.

†Ulcerative, necrotic also known as ecthyma gangrenosum.

are sinusitis and pneumonia although invasion of adjacent structures and dissemination may occur in the setting of profound and prolonged immunosuppression. Primary cutaneous and gastrointestinal infections are very rare.

## Other mycoses

### *Fusarium* spp

*Fusarium* is the second most common opportunistic mould after *Aspergillus* spp. in severely immunocompromised cancer patients.<sup>18</sup> Infections in high-risk cancer patients include sinusitis, pneumonia, extremity cellulitis (skin breakdown), and disseminated infection. Metastatic skin infections and fungemia are features that help differentiate fusariosis from aspergillosis.

Among HSCT recipients, the incidence of fusarial infection is around 5.9 cases per 1000 transplants and varies according to type of transplant: 1.4–2.0 in autologous HSCT, 2.3–5.0 in matched related donor transplants, and 20 cases per 1000 transplants in mismatched related donor transplants.<sup>88</sup> Among non-transplant recipients, the infection is most common among patients with AML undergoing remission induction therapy. The clinical syndromes associated with invasive fusariosis in cancer patients are discussed in Chapter 13. Risk factors for fusariosis in high-risk cancer patients include severe and prolonged neutropenia after conditioning therapy.<sup>18</sup> Multiple myeloma and prolonged neutropenia have also been identified as risks for fusariosis in one study.<sup>88</sup> Outcome of fusarial infections remains very poor<sup>18</sup> except in a subset of patients, those whose hematopoietic function recovers, and those who have limited rather than disseminated disease, limited GvHD, and no corticosteroid therapy.<sup>88,106</sup>

Most infections are caused by *F. solani*, followed by *F. moniliforme* and *F. oxysporum*.<sup>18</sup>

Geographic differences appear to exist in the incidence of fusariosis, with a cancer center in Texas reporting increased infections<sup>18</sup> compared to fewer infections in most others.<sup>87</sup>

As with aspergillosis, nosocomial acquisition of severe fusariosis from the hospital water supply of a cancer center in Texas has been documented.<sup>79</sup> Refer to Chapter 13 for additional information on fusariosis.

### Zygomycetes

Most infections in cancer patients are caused by *Rhizopus* spp., *Mucor* spp., *Absidia* spp., and *Rhizomucor* spp., and more recently by *Cunninghamella* spp.<sup>107</sup> These infections originate in the sinopulmonary tract and may progress locally or disseminate,

although a gastrointestinal tract mode of acquisition with contiguous extension into nearby structures is well known.

The incidence of zygomycosis in high-risk cancer patients is variable, ranging from <1% to 2.5%, with some reports suggesting a recent increase in frequency.<sup>30-37</sup>

Risk factors are similar to those of aspergillosis and include severe, prolonged neutropenia and corticosteroid therapy. Poorly controlled diabetes mellitus is another known risk factor for this infection. Among HSCT recipients, zygomycosis also occurs late after engraftment.

A rhinocerebral presentation is the most typical form of zygomycosis in diabetic patients, but is less common among neutropenic cancer patients who tend to develop pneumonia, sinusitis or cutaneous infections. Among HSCT recipients, disseminated and rhinocerebral patterns of infection may also develop. Please refer to Chapter 12 for additional information on zygomycosis.

### *Scedosporium* spp

In immunosuppressed patients, *Scedosporium* species (*S. apiospermum* more commonly than *S. prolificans*) cause diseases similar to aspergillosis, including sinusitis, pneumonia and cutaneous infections.<sup>108,109</sup> Fungemia is not uncommon and distinguishes scedosporiosis from aspergillosis.

Geographic differences exist in the distribution of infections caused by *Scedosporium* species (higher incidence of *S. prolificans* in Spain, Australia, and parts of the United States).

Outcome of infection is very poor if immunosuppression is not reversed. Further complicating this issue is the resistance of *S. prolificans* to all available antifungal agents.<sup>110,111</sup> Please refer to Chapter 13 for additional information on scedosporiosis.

### *Cryptococcus neoformans*

Infections caused by *Cryptococcus neoformans* are now rare in cancer patients and occur almost exclusively in patients with chronic hematologic malignancies, usually in the setting of prolonged deficiency in CMI.<sup>112</sup> Meningitis is the most common clinical presentation, with pulmonary and skin manifestations occurring less frequently. *Cryptococcus laurentii* has emerged as an important cause of fungemia in neutropenic patients, and peritonitis in a setting of continuous ambulatory peritoneal dialysis.<sup>113</sup>

Occasional reports have described *C. albidus* sepsis and meningitis, *C. curvatus* myeloradiculitis, and *C. humicola* meningitis.<sup>114</sup>

Please refer to Chapter 9 for additional information on cryptococcosis.

**Table 20-8** Agents of hyalohyphomycosis and phaeohyphomycosis known to cause invasive disease in cancer patients

Hyalohyphomycosis	Phaeohyphomycosis
<i>Acremonium</i> spp.	<i>Alternaria</i>
<i>Aspergillus</i> spp.	<i>Aureobasidium</i>
<i>Chrysosporium</i> spp.	<i>Bipolaris</i> spp.
<i>Fusarium</i> spp.	<i>Chaetomium</i> spp.
<i>Paecilomyces</i> spp.	<i>Cladophialophora</i>
<i>Penicillium marneffe</i>	<i>Cladosporium</i>
<i>Scedosporium apiospermum</i>	<i>Coniothyrium</i>
<i>Scopulariopsis</i> spp.	<i>Curvularia</i>
<i>Trichoderma</i> spp.	<i>Dactylaria</i>
	<i>Exophiala</i>
	<i>Exserohilum</i>
	<i>Fonsecaea</i>
	<i>Lasiodiplodia</i>
	<i>Lecythophora</i>
	<i>Phaeoacremonium</i>
	<i>Phialemonium</i>
	<i>Phialophora</i>
	<i>Phoma</i>
	<i>Ramichloridium</i>
	<i>Rhinocladiella</i>
	<i>Scedosporium prolificans</i>
	<i>Scytalidium</i>
	<i>Wangiella</i>

## Other less common fungal opportunists

Yeasts such as *Blastoschizomyces capitatus*, *Trichosporon asahii*, *Saccharomyces cerevisiae*, *Rhodotorula* spp., *Malassezia furfur* and other yeasts may cause infections in cancer patients<sup>115</sup> and are discussed in Chapter 10. The agents of hyalohyphomycosis and phaeohyphomycosis (Table 20-8; Chapters 13 and 14) are also increasingly reported among cancer patients.<sup>19,116-118</sup>

The endemic mycoses, *Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, and *Penicillium marneffe*, may occasionally cause infection in cancer patients, following travel to or residence in endemic regions.<sup>119,120</sup> In general, however, these agents infrequently cause disease, even within endemic regions. Please refer to Chapter 15 for additional information.

## Diagnosis of invasive fungal infections in cancer patients

### General principles

An early diagnosis of IFIs is critical to the successful outcome of cancer therapy because of the high risk for and serious consequences of these infections in cancer patients. These consequences may be direct, including significant morbidity and mortality due to higher microbial loads in severely

immunosuppressed patients, extensive treatment with its attendant toxicity, cost and development of resistance, and potential delays in therapy of the underlying cancer. Indirect consequences of IFIs may also be severe such as in allogeneic HSCT recipients in whom infections may initiate GvHD.

Unfortunately, delayed diagnosis is common, implying that start of therapy is late in the course of infection. At this stage, the microbial load, a key determinant of prognosis, may be too high for a successful outcome, particularly as infected patients are usually severely immunosuppressed and lack the protective defenses almost always required for response to therapy.

Challenges in the early diagnosis of infection in cancer patients are due to the following three factors.

1. Severe myelosuppression and immunosuppression.
  - A broader spectrum of pathogens with clinical presentation similar to those encountered among individuals with intact immunity
  - Subtle or atypical presentation of infection; impaired inflammatory response among severely immunocompromised hosts renders clinical presentation more subtle and radiologic findings less diagnostic than in mildly immunosuppressed or immunocompetent individuals with similar pathogens and microbial load.
  - Simultaneous and sequential infection in these patients is common,<sup>21,25,93,103</sup> further complicating diagnosis.
  - Inability to distinguish between pathogens on the basis of clinical, radiologic or histopathologic findings only, such as the angioinvasive moulds, all of which share three cardinal features: hemorrhage, infarction, and metastases.
  - Faster progression of the clinical course of infection.
2. Confounding variables that are particular to cancer patients.
  - Non-infectious causes of fever (tumor fever, engraftment syndrome, GvHD and others)
  - Non-infectious causes of pulmonary infiltrates (engraftment syndrome, toxicity of antineoplastic agents and others)
  - Immune reconstitution: rapid immune reconstitution following resolution of neutropenia is associated with transient clinical and radiologic deterioration. The pulmonary immune reconstitution inflammatory syndrome (PIRIS) of IPA is typically, but incorrectly, considered evidence of progressive infection on the basis of which patients may undergo additional diagnostic procedures and treatment modifications including enrollment on investigational agents. These patients are in fact achieving control of their infection and do not require any treatment modifications.<sup>121</sup>
3. Limited diagnostic options.
  - Inability to obtain diagnostic samples such as biopsies of difficult-to-reach infected sites because of the frequency of severe thrombocytopenia and coagulopathies in these critically ill patients.
  - Limited diagnostic tests: many serodiagnostic tests have long been available for the etiologic agents of the endemic mycoses in the general population, in contrast to the paucity of diagnostic tests in cancer patients.

Because of these difficulties in securing a timely diagnosis in cancer patients, broad empiric antifungal therapy is usually

applied, often with significant toxicity that may involve interactions with antineoplastic and immunosuppressive agents.

Early and specific microbiologic diagnosis in cancer patients is therefore critical for guiding treatment and minimizing non-essential drug therapy, and invasive diagnostic procedures may be required.

## Diagnostic tools

### Microbiologic tests

Chapter 3 provides detailed information on laboratory methods for fungal diagnosis. These include direct examination of wet mounts and tissues using various stains and techniques, cultures and non-culture diagnostic tests.

It is important to emphasize that identification of the fungal pathogen by culture or molecular tools is critical because of the difficulties in distinguishing infections caused by fungi with similar properties, such as the angioinvasive moulds. Indeed, it is practically impossible to distinguish infection with *Aspergillus* species from infection by any of the angioinvasive moulds such as *Fusarium*, *Scopulariopsis* and *Scedosporium* spp., or the dematiaceous fungi such as *Bipolaris*, *Exserohilum* and *Alternaria* species and, to a lesser extent, infections caused by the agents of zygomycosis. Table 20-9 summarizes the microscopic features of important pathogens and Table 20-10 briefly describes the role of serodiagnosis in pertinent infections.

In this chapter, we will limit our discussion to two non-culture tests that are of particular importance to cancer patients, namely the detection of galactofuranosyl-containing molecules (*Aspergillus* galactomannan antigen test) and of  $\beta$ 1,3-d-glucan.

Detection of galactofuranosyl-containing molecules Detection of *Aspergillus* antigens in blood using the Bio-Rad Platelia™ sandwich ELISA has gained widespread acceptance as a sensitive method for the early diagnosis of aspergillosis<sup>122,123</sup> and is now included in the consensus criteria for defining IFIs in cancer patients and HSCT recipients (EORTC/MSG criteria).<sup>124</sup>

The test has excellent performance characteristics in profoundly neutropenic patients, provided it is performed at least 2–3 times a week and supported by chest CT scan findings (usually taken in patients with >4–5 days of persistent neutropenic fever).<sup>125</sup> Limitations of the test include reduced sensitivity during receipt of mould-active antifungal agents and false-positive results with the use of some semi-synthetic  $\beta$ -lactam antibiotics<sup>126–130</sup> (including ampicillin, amoxicillin-clavulanate and piperacillin-tazobactam) and gluconate-containing plasma expanders.<sup>131</sup> Despite these limitations, the excellent test performance of the Platelia™ sandwich ELISA in neutropenic patients makes it a particularly useful diagnostic test for aspergillosis.<sup>132</sup> Other pathogenic fungi can also test positive for galactomannan<sup>133</sup> (Table 20-11). Concerns about a paradoxical effect with this test do not appear to be clinically important.<sup>134</sup>

Furthermore, the kinetics of the Platelia™ sandwich ELISA test correlate with aspergillosis outcome<sup>135,136</sup> and may allow differentiation of progressive aspergillosis from the recently described PIRIS that develops during neutrophil recovery in patients who are achieving microbiologic control of aspergillosis and who ultimately recover without any changes in antifungal therapy.<sup>121</sup>

Detection of  $\beta$ 1,3-d-Glucan  $\beta$ 1,3-d-glucan assays are widely used in Japan and the Fungitell™ assay has recently been

approved by the US FDA based on studies in patients with acute leukemia.<sup>137,138</sup> The negative predictive value of twice-weekly sampling is 100% and test results are not influenced by the use of mould-active antifungal agents. However, false-positive readings may result from the use of albumin or immunoglobulins, exposure to glucan-containing gauze, hemodialysis, and some antimicrobial preparations (amoxicillin-clavulanate).<sup>138</sup> The  $\beta$ 1,3-d-glucan assay can detect a much broader spectrum of fungal species than the Platelia™ sandwich ELISA, including *Candida*, *Aspergillus*, *Fusarium* and others but not *Cryptococcus* or the agents of zygomycosis. Using the combination of Platelia™ sandwich ELISA and the  $\beta$ 1,3-d-glucan assay promises to be particularly useful in cancer patients at risk for IFI.<sup>139,140</sup>

An important development related to the presence of  $\beta$ 1,3-glucan in the cyst wall of *Pneumocystis jiroveci*<sup>141</sup> is the significant role of the plasma  $\beta$ 1,3-d-glucan test as a diagnostic and prognostic test in *P. jiroveci* pneumonia (PJP).<sup>142–148</sup>

### Imaging

In neutropenic patients, CT scan of the chest should be performed at the first suspicion of IPA: unexplained or relapsing fever; suggestive clinical signs/symptoms (dyspnea, non-productive cough, pleuritic chest pain and a pleuritic friction rub); isolation of *Aspergillus* spp. or other opportunistic mould or positive non-culture based assays.<sup>125,149</sup>

The radiologic findings may be greatly influenced by the net state of immunosuppression. In general, however, the differential diagnosis can be aided by a few imaging patterns that develop in certain settings (patients at risk with a compatible clinical picture).

1. In a neutropenic patient with a hematologic cancer: >, 1 cm pulmonary nodules, with or without halo signs on CT scan of the chest, caused by angioinvasive opportunistic fungi (*Aspergillus*, *Fusarium*, zygomycetes, other).<sup>150–153</sup>
2. In a patient with a hematologic cancer following neutrophil recovery.
  - Pulmonary nodule with cavitation and/or air crescent signs caused by angioinvasive opportunistic fungi.
  - Multiple hepatic, splenic (rarely kidney) nodules in association with chronic disseminated yeast infection (candidiasis, other).<sup>154–156</sup>
3. In a patient with severe T cell immunodeficiency.
  - Diffuse miliary lung nodules and/or intraabdominal abnormalities due to disseminated endemic mycoses.
  - Cerebral nodules, enhancing cerebral gyri, and/or diffuse miliary or focal lung lesions due to *Cryptococcus neoformans*.

It should also be underscored that the CT findings suggestive of angioinvasive fungal infections may also occur with bacterial, viral, mycobacterial, and parasitic infections. Because the clinical conditions favorable for the development of IFI also facilitate other pathogenic microorganisms, these radiologic findings may also be caused by non-fungal pathogens or by polymicrobial infection (fungal and other).<sup>152</sup>

In addition to CT scan and MRI of various organs, positron emission tomography (PET) with CT scan (PET/CT) has recently been shown to be particularly useful in the diagnosis and management of various infections in cancer patients, including IFI.<sup>157–159</sup>



**Table 20-9** Characteristic features of fungi that cause invasive infection in cancer patients

Pathogen	Yeasts	Hyphae	Pseudohyphae	Blastoconidia	Arthroconidia	Aleurioconidia	Capsule
<i>Candida</i> and other yeasts	+	+♦	+	+	–	–	–
<i>Trichosporon</i> spp.	+	+♦	+	+	+	–	
<i>Blastoschizomyces capitatus</i>	+	+♦	+		+	–	
<i>Malassezia</i> spp.	+	+/–	–	–	–	–	
<i>Cryptococcus neoformans</i>	+	–	–	–	–	–	+
<i>Aspergillus</i> spp.		+♦				+ <i>A. terreus</i>	
Hyalohyphomycosis	+	+♦	+			+ other fungi §	
Phaeohyphomycosis	+ (variable)	+♦	+				
<i>Penicillium marneffei</i>	+ at 37°C (budding absent)	+ at 25°C					
Zygomycetes	–	+▲	–				
<i>Histoplasma capsulatum</i>	+ at 37°C budding, within macrophages	+ at 25°C					
<i>Blastomyces dermatitidis</i>	+ at 37°C Broad-based, budding	+ at 25°C	+				+
<i>Coccidioides immitis</i>	+ endoconidia in spherules	+			+		
<i>Paracoccidioides brasiliensis</i>	+						
<i>Sporothrix schenckii</i>	+ ‡	+ rare at 25°C					
<i>Pneumocystis jiroveci</i>	–	–	–	–	–	–	–

♦Hyphae, 2.5–7 µm, uniform with dichotomous branching, septate, parallel walls.  
▲Hyphae: large 3–25 µm, rare septation, irregular branching, non-parallel walls. Distortion of hyphal structures common.  
§Aleurioconidia present: *Aspergillus terreus*, *Fusarium* spp., *Paecilomyces* spp., *Acremonium strictum*, *Scedosporium prolificans*.  
‡Sporotrichosis: variable size and shape from globose to ovoid yeasts ("cigar-shaped"). Tissue reaction forms asteroid bodies.

**Table 20-10** Diagnostic immunology testing for invasive fungal infections

	Test	Diagnosis	Monitor response	Limitations	Comments
YEASTS					
<i>Candida</i> spp.	βDG	+ βDG	-	Limited data	βDG promising. Serial values and combinations of tests may be useful
<i>Trichosporon</i> spp.	βDG, Crypto Ag	+ βDG	-	Limited data	
<i>Saccharomyces</i> spp.	βDG	+ βDG	-	Limited data	
<i>Cryptococcus neoformans</i>	Crypto Ag	+ serum, CSF, pleural fluid	+/- Titer correlates with disease burden but less useful to monitor response	Crypto Ag may be (+) in infections by <i>Trichosporon</i> spp., <i>Capnocytophaga canimorsus</i> , <i>Stomatococcus</i>	If serum titer positive, sample other sites (CSF, pleural fluid) even in the absence of symptoms
MOULDS					
<i>Aspergillus</i> spp.	Asp. GM Ag	+	+	Asp. GM may be falsely (+) with certain antibiotics and gluconate containing solutions	Twice-weekly serial monitoring for diagnosis. More frequent testing to monitor rapidity of response.
				Asp. GM cross-reacts with other galactomannan-containing organisms (see Table 20-11)	
	βDG	+	-	Limited data	
<i>Fusarium</i> spp.	βDG	+	-	Limited data	
Other moulds	βDG		-	Limited data	
DIMORPHIC	Asp. GM Ag	+	?	Limited data	
<i>Histoplasma capsulatum</i>					
Low disease burden ♦	Ab (ID, CF)	Serum, urine	+		Order serum Ab (ID and CF) and serum, urine and site-specific Ag.
High disease burden ♦	Ag + Ab (ID, CF)	Urine, serum, site-specific (CSF, BAL)	+		Order serum Ab (ID and CF) and serum, urine and site-specific Ag.

**Table 20-10** Diagnostic immunology testing for invasive fungal infections—cont'd

	Test	Diagnosis	Monitor response	Limitations	Comments
Blastomycosis	Ag	Urine	?	Limited data	
Coccidioidomycosis	Ab (several) §	Serum (IDTP, IDCF). CF for specimens other than serum. §	+	Antibodies may take months to develop and may never develop in immunocompromised patients. Negative Ab does not exclude infection	Order serum (IDTP or IDCF) and CF for specimens other than serum. §
Paracoccidioidomycosis	Ab, Ag	Serum, urine and site-specific	+	Antibodies may never develop in immunocompromised patients. Negative Ab does not exclude infection or differentiate disease activity	Order serum Ab and serum, urine and site-specific Ag.

Testing is for serum unless otherwise specified; Crypto Ag, cryptococcal antigen; BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid; ID, immunodiffusion; CF, complement fixation. βDG, β1,3-d-glucan; Asp. GM, *Aspergillus* galactomannan; Asp. GM cross-reacts with other galactomannan-containing organisms (see Table 20-11). Ag, antigen; Ab, antibody; DA, d-arabinitol.

§Coccidioidomycosis antibody tests: tube precipitin, complement fixation (CF), immunodiffusion using tube precipitin (IDTP), immunodiffusion using complement fixation antigen (IDCF), enzyme immunoassay, latex agglutination. IDTP and IDCF most frequently used. CF used for specimens other than serum (CSF, pleural fluid, synovial fluid, etc.).

Panfungal βDG: *Candida*, *Aspergillus*, *Trichosporon*, *Fusarium*, *Saccharomyces* but NOT *Cryptococcus* or the agents of zygomycosis.

◇*H. capsulatum* disease burden: high, acute diffuse pulmonary or meningitis; low, acute pulmonary, localized or chronic.

Chapter 4 provides detailed information on the radiologic methods for diagnosing IFIs.

## Clinical manifestations and diagnosis

### General principles

The clinical manifestations of IFI in cancer patients vary widely and depend on three factors:

- the site of infection (lungs, sinuses, systemic, other)
- its pathogenesis (angioinvasive or not) and
- the severity and dynamic nature of immunosuppression.

As an example, IPA in neutropenic patients tends to be associated with early clinical findings of pneumonia such as dyspnea, cough, and pleuritic chest pain, so that IPA would be unlikely in the absence of such findings. In contrast, these symptoms are less likely among HSCT recipients in whom infection may often present as unexplained fever, until later on when symptoms

indicative of a localized infection develop. Similarly, responding IPA in neutropenic patients may develop worsening clinical and radiologic findings coinciding with neutrophil recovery, as a manifestation of immune reconstitution (PIRIS).<sup>121</sup>

### Unexplained Fever

Because of immunosuppression, cancer patients cannot mount an adequate inflammatory response to pathogens. As a result, signs and symptoms and radiologic findings of IFI may be muted, leading to significant delays in diagnosis; more than one-third of patients with IFI diagnosed at autopsy never received antifungal therapy.<sup>160</sup> Following immune reconstitution, the clinical and radiologic findings may become apparent, reflecting the recovering immune system's attempt at controlling the infection which is at times possible<sup>121</sup> but not always, in which case deterioration reflects progressive infection.<sup>161</sup> Other than fever, few other clinical signs exist to alert the clinician to the possibility of an IFI (see Tables 20-5 to 20-7) although chronic disseminated candidiasis and the similar syndromes caused by *Trichosporon* spp.



**Table 20-11** Cross-reactivity of the *Aspergillus* galactomannan antigen tests

Organism	Human infection	In vitro susceptibility	Comment
<b>HYALINE MOULDS</b>			
<i>Fusarium</i> ( <i>oxysporum</i> , <i>solani</i> )	Yes	<i>F. oxysporum</i> may be susceptible to voriconazole, posaconazole. For <i>F. solani</i> , use high-dose lipid amphotericin B	In vitro susceptibility may be helpful
<i>Paecilomyces</i> ( <i>lilacinus</i> , <i>variotii</i> )	Yes	Susceptible to amphotericin B, ( <i>P. variotii</i> ) and the mould-active triazoles*	
<b><i>Penicillium</i></b>			
<i>P. marneffei</i>	Yes	Susceptible to amphotericin B, and the mould-active triazoles*	
<i>P. chrysogenum</i>	Yes	Susceptible to amphotericin B, and the mould-active triazoles*	
<i>P. digitatum</i>	No	Not applicable	
<i>Trichothecium roseum</i>	No	Not applicable	Dermatophytosis in animals
<b>DEMATIACEOUS MOULDS</b>			
<i>Acremonium</i> spp., <i>Alternaria</i> spp., <i>Wangiella dermatitidis</i>	Yes	Susceptible to amphotericin B, and the mould-active triazoles*	
<i>Nigrospora oryzae</i>	No	Not applicable	
<b>DIMORPHIC FUNGI</b>			
<i>Histoplasma capsulatum</i>	Yes	Susceptible to amphotericin B, and the mould-active triazoles*	<i>Aspergillus</i> GM positive in disseminated infection
<i>Blastomyces dermatitidis</i>	Yes	Susceptible to amphotericin B, and the mould-active triazoles*	
<b>YEASTS</b>			
<i>Cryptococcus neoformans</i>	Yes	Susceptible to amphotericin B, and all triazoles	
*mould-active triazoles: itraconazole, voriconazole, posaconazole			

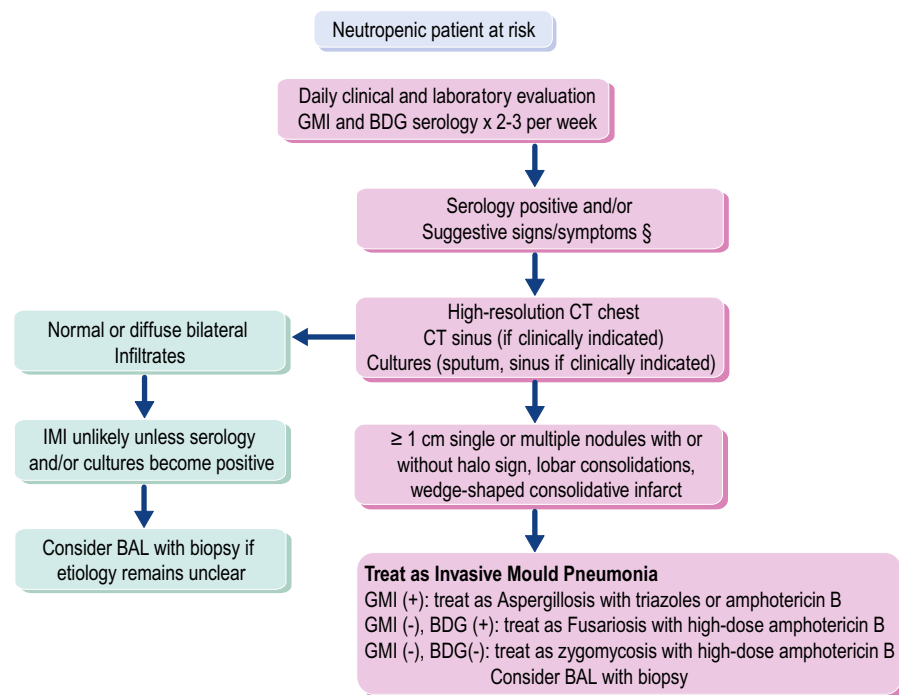
and *Blastoschizomyces capitatus* infections present with particularly characteristic findings following neutrophil recovery. Ophthalmologic examination is a potentially useful tool for monitoring patients at risk of disseminated infection, particularly candidiasis, and can establish infection in patients with negative blood cultures. However, endophthalmitis rarely occurs in neutropenic patients because the lesions result from an inflammatory response that requires neutrophils.

## Pneumonia (Figure 20-1)

Pneumonias in cancer patients are caused by many pathogens, particularly bacterial. However, a localized pulmonary infiltrate, progressing despite broad-spectrum antibacterial

antibiotics, particularly if associated with persistent fever, dyspnea, non-productive cough, pleuritic chest pain and a pleuritic friction rub, should be considered as caused by IPA or by other IMI until proven otherwise. Because the clinical and radiologic features of IPA and IMI of the lungs are very similar, they will be discussed together. A clinical picture of thromboembolism with infarction (pulmonary but also cerebral, myocardial or other) should further raise the index of suspicion for IPA because of the angioinvasive nature of these moulds.

A chest CT scan should be performed at first suspicion of IPA in neutropenic patients and may show the halo sign, an early finding suggestive of angioinvasive pulmonary mycosis and which consists of single or multiple >1 cm nodules surrounded by a halo of lower attenuation. Other suggestive



CT: computerized tomography; GMI: serum aspergillus galactomannan index; BDG: serum B-D-Glucan; BAL: bronchoalveolar lavage; IMI: invasive mould infection  
§ Fever, unresponsive to broad spectrum antibiotic; pleuritic chest pain; pleural rub

**Figure 20-1** Management of a patient with cancer and pulmonary infiltrates who is at risk for an invasive mould pneumonia..

findings include >1 cm pulmonary nodules without halo sign, consolidations and wedge-shaped consolidative infarcts. Ground-glass opacities, bronchiolitis-bronchopneumonia pattern, and pleural, pericardial, hilar and mediastinal lesions also occur in patients with IPA but have no discriminative power.

Upon marrow recovery, the pulmonary lesions of IPA become smaller and many will resolve; some, however, cavitate with or without air crescent formation and ultimately resolve with response to therapy. In patients who fail to respond to therapy, the lesions may enlarge and newer infiltrates may develop, with progressive respiratory failure and death. Rarely, hyphae invade adjacent large vascular structures, causing thrombosis and pseudoaneurysm with risk of rupture and fatal hemoptysis, particularly when neutrophil recovery is rapid.<sup>162</sup>

The clinical and radiologic course of IPA varies according to the net state of immunosuppression.<sup>162,163</sup> In severely immunosuppressed patients, IPA usually causes invasive and rapidly progressive bronchopneumonia in contrast to the more indolent course and localized infection in mildly immunosuppressed patients. As a result of the dynamic state of immunosuppression, one form of the disease may progress into another, in a bidirectional fashion.

Chronic tracheobronchial aspergillosis may develop in some patients in whom the airway spread of aspergilli causes substantial bronchial damage (wall thickening and nodularity, bronchostenosis, and bronchiectasis), leading to peribronchial consolidation and atelectasis.<sup>164,165</sup> When *Aspergillus* spp. invade large bronchi, they may cause extensive ulceration, although *Aspergillus* tracheobronchitis is uncommon in cancer patients.<sup>166</sup> Chest radiographs and CT scans are usually normal in this setting despite the presence of respiratory symptoms.

Hematogenous candidiasis is associated with pulmonary lesions which are often bilateral and diffuse, including >1 cm nodules,<sup>167</sup> diffuse numerous miliary nodules (1–3 mm) or a bronchiolitis-bronchopneumonia pattern.<sup>168</sup> The presence of concomitant disseminated lesions in liver, spleen, and/or kidneys on abdominal CT favors the diagnosis of hematogenous candidiasis with pulmonary seeding.

Fever, cough, and chest pain and bilateral infiltrates are findings common to most pneumonias, including with fungi such as *Cryptococcus neoformans* and the agents of the endemic mycoses. *Pneumocystis jiroveci*, now classified as a fungus, is the predominant cause of interstitial pneumonia in HSCT recipients not receiving prophylaxis. Progressive dyspnea with fever and malaise followed by non-productive cough and bilateral infiltrates are the typical findings.

A critical decision facing the clinician caring for cancer patients with pulmonary infiltrates is whether or not to undertake invasive diagnostic procedures. Sputum cultures are rarely diagnostic and the yield of bronchoscopy with bronchoalveolar lavage (BAL) is disappointingly low,<sup>169</sup> except in patients with *Pneumocystis jiroveci* pneumonia (PJP) or with one of the endemic mycoses. Percutaneous needle aspiration improves the quality of specimens for histologic examination and/or culture but may not be possible because of coagulopathies. Fortunately, the recent successful application of serum<sup>122,123</sup> and BAL Platelia™ sandwich ELISA<sup>170-172</sup> and the serum  $\beta$ 1,3-d-glucan assay<sup>137</sup> has greatly simplified and improved the diagnostic yield. Notably, the serum  $\beta$ 1,3-d-glucan assay is also useful for the diagnosis of PJP.<sup>142-148</sup> Figure 20-1 provides a diagnostic strategy for the pulmonary mycoses in cancer patients.

## Sinonasal syndromes

Sinusitis caused by *Aspergillus* spp. (and less frequently by other moulds) is frequently limited to the paranasal sinuses (most commonly, the maxillary sinuses) although extension into the orbits or the brain may occur with severe immunosuppression. Clinical manifestations may be limited to facial pain but may progress to include palatal or orbital cellulitis with subsequent ulceration of the hard palate, which can be seen on examination.

A rhinocerebral clinical presentation is seen with the zygomycetes and may be particularly severe unless immediate and aggressive therapy is started. Unlike other forms of fungal sinusitis in which the infection starts in the paranasal sinuses, rhinocerebral zygomycosis starts in the nasal cavity with subsequent spread to the adjacent paranasal sinuses. Presentation includes facial pain and swelling, ptosis, proptosis, and dilatation or fixation of the pupil, and a dark, serosanguinous nasal or ocular discharge, palatal or orbital cellulitis which may progress to ulceration with necrosis or perforation of the nasal septum and other nearby structures.

Progression of sinonasal fungal infections to the surrounding structures including the carotid artery, orbit, cavernous sinuses and cranial nerves and brain can be devastating. CT scan shows sinus opacification and may detect bone erosion and extension into the cavernous sinus, brain or orbit. Sinus opacification may, however, simply represent thick pus, hemorrhage, desiccated mucosal secretions or dystrophic calcifications<sup>173,174</sup> and MRI with or without gadolinium is recommended if the diagnosis is uncertain or when intracranial extension or vascular involvement is suspected. Air-fluid levels are rare in fungal sinusitis.

## Central nervous system infections

Involvement of the central nervous system (CNS) by IFI may occur most commonly through hematogenous spread (*Candida*, other yeasts, moulds) or through direct extension from a sinonasal infection (moulds).<sup>175</sup> Fungi associated with CNS involvement include *Candida* spp., *Cryptococcus neoformans*, *Aspergillus* spp., the agents of hyalohyphomycosis and rarely the agents of phaeohyphomycosis<sup>175</sup> (see Table 20-8). For additional details on dematiaceous fungi, please refer to Chapter 14.

The presentation of CNS infections depends on the site of origin, the localization of the infection, and the net state of immunosuppression. If the infection is from direct extension from the sinuses, symptoms may include orbital swelling, facial pain, and nasal congestion in addition to headache, seizures or other focal neurologic signs. Hematogenous dissemination, on the other hand, can lead to a variety of findings depending on the CNS sites involved.

Chapter 6 describes the radiographic findings of CNS mycoses. Similar to pulmonary lesions, brain lesions of aspergillosis (and other IMIs) appear initially as well-defined macronodular hypodensities or large vessel infarcts surrounded by edema on CT and as T2-weighted hyperintensities on MRI surrounded by edema that enhance after intravenous gadolinium. Central necrosis with rim enhancement develops later. Three patterns of cerebral aspergillosis in cancer patients are observed: infarctions (with or without hemorrhage), abscesses, and dural enhancement in the adjacent paranasal sinuses, orbit or skull.

Cerebral candidiasis in adults most commonly results in multiple microabscesses, solid enhancing lesions or granulomas and rarely in meningitis or encephalitis. Occasionally, vascular invasion by *Candida* may lead to thrombosis with infarction (with or without hemorrhage), vasculitis, and mycotic aneurysms. Cerebrospinal fluid examination is diagnostic in <50% of cases.

*Cryptococcus neoformans* of the CNS presents most commonly as meningitis. As with candidiasis, nodules, microabscesses, solid enhancing lesions or granulomas may be seen.

Phaeohyphomycosis of the CNS usually presents as abscesses or as a large lesion with mass effect while various manifestations are seen with the endemic mycoses, including meningitis, encephalitis, abscesses (blastomycosis and coccidioidomycosis) or as solid enhancing lesions or granulomas (histoplasmosis and paracoccidioidomycosis).

## Skin and soft tissue infections

Skin and soft tissue involvement by fungi in cancer patients can manifest in two forms: primary or secondary (metastatic) lesions. Primary lesions result from direct (usually traumatic) inoculation of fungi at various sites, including at the site of insertion of central venous catheters (CVC). In neutropenic patients, the infection starts as skin erythema and induration at the puncture site and progresses to necrosis with tissue infarction and vascular invasion, with potential dissemination to other organs. Most are accounted for by *Aspergillus* and *Fusarium* spp.<sup>176</sup>

Secondary lesions result from hematogenous dissemination and occur with yeast infections (*Candida* and *Trichosporon* spp. and *Blastoschizomyces capitatus*) and infections caused by moulds (*Aspergillus* or *Fusarium* spp., rarely with the zygomycetes). Lesions can be nodular, maculopapular, pustular or ulcerative with central necrosis (ecthyma gangrenosum) or even resembling purpura fulminans (see Table 20-7).

A particular note should be made about extremity cellulitis at the site of skin breakdown (onychomycosis, other). This presentation is typically observed with fusariosis though it may occur with other IMIs. Lymphangitic spread may be observed and heralds dissemination to various sites (see Chapter 13).

Because IFI are notoriously difficult to diagnose, and because cutaneous lesions are easy to sample and offer a high diagnostic yield, a complete physical examination should be performed daily in a search for cutaneous and subcutaneous lesions.<sup>177</sup> Biopsies should be submitted for microbiologic, histopathologic, immunohistochemical, and molecular testing.

## Gastrointestinal and other clinical syndromes

Gastrointestinal involvement by opportunistic fungi usually results from hematogenous seeding and is typically recognized at autopsy examination in patients with severe immunosuppression. When symptomatic, the infection manifests as typhilitis or enterocolitis (fever, abdominal pain). Life-threatening hematochezia may develop. Bowel obstruction, ischemia, infarction and perforation are rare and usually late events.<sup>178-182</sup>

Primary gastrointestinal infection is extremely rare and may result from the ingestion of a large fungal inoculum in a severely immunosuppressed patient.



Uncommon manifestations of IFIs in cancer patients include peritonitis in patients undergoing continuous ambulatory peritoneal dialysis, CVC-related infection, otitis, endophthalmitis, osteomyelitis and septic arthritis, native and prosthetic valve endocarditis, and isolated involvement of practically any organ.

## Management of fungal infections in cancer patients

### General principles

#### 1. Identify and treat all fungal infections prior to commencing antineoplastic therapy

Pretreatment screening is designed to identify and treat pre-existing or developing infections. Preexisting infections, typically acquired following antineoplastic therapies, should be aggressively treated with intention to cure prior to initiating cytotoxic therapies because of the serious risk of exacerbation of these infections as a result of additional myelosuppression/immunosuppression.

In patients with candidiasis, surgery is indicated for endocarditis and for removal of infectious foci. Resecting necrotic tissue in patients with IMIs may help to control the infection and reduce the risks of relapse and of potentially fatal hemoptysis which is likely in patients whose lesions are close to the pulmonary artery.<sup>183-185</sup>

#### 2. Reduce exposure to fungal pathogens in the healthcare setting and the community

Measures to reduce host exposure to fungal pathogens in healthcare settings include the following.

- Strict hand-washing precautions (potential transmission of candidiasis on the hands of healthcare workers).<sup>186</sup>
- Installation and appropriate maintenance of specialized air filtration units to minimize exposures of high-risk patients to potential sources of airborne fungi such as *Aspergillus* spp. and others.<sup>187-189</sup>
- Avoidance of exposure to hospital tap water,<sup>39,41,79</sup> and cleaning water-related structures in patients' bathrooms.<sup>40</sup>
- The use of high-efficiency masks during patient transport to other settings is a standard recommendation in hospital facilities housing susceptible patients.
- Staff education.
- Targeted surveillance in high-risk patients.
- Investigation of potential sources of fungal infection (including molecular relatedness studies) if new cases are diagnosed. The index of suspicion should be raised for the presence of an intense environmental exposure when a serious fungal infection develops in a patient whose net state of immunosuppression is not severe enough to be otherwise associated with this infection.

Avoidance of activities that increase the risk of exposure to pathogenic fungi in the community setting is strongly recommended during periods of severe immunosuppression (see Table 20-2 for the activities associated with specific IFIs).

#### 3. Prevent GvHD in allogeneic HSCT recipients

This relies on careful donor evaluation, optimal tissue typing and matching, and individualization and close monitoring of immunosuppressive regimens.

#### 4. Enhance immunity

A cardinal rule of therapy in immunosuppressed patients with IFIs is the reduction of immunosuppressive therapies and resolution of immunosuppressive conditions, because these represent the most important risk factors for developing IFIs and for failure to respond to antifungal therapy. Strategies to improve immunity include the following.

- Judicious use of immunosuppressive agents; the practice of starting large doses of corticosteroids for suspected GvHD should be reserved to life-threatening conditions. When possible, it is advisable to document a diagnosis of GvHD (biopsies, cytokine measurements, other) prior to commencing corticosteroids.
- Reduce dose of immunosuppressants when possible.
- Use colony-stimulating factors (CSF) and interferon- $\gamma$ .<sup>190</sup>
- Transfuse granulocytes elicited with granulocyte-CSF and corticosteroids in severely neutropenic patients.<sup>191-193</sup>
- Iron chelation: in patients with severe iron overload, removal of iron may also improve organ function.<sup>51,73</sup>

#### 5. Improve organ function

Proper care of prosthetic devices and the avoidance of potentially nephrotoxic-hepatotoxic agents and conditions may help reduce the risk of IFI. Keratinocyte growth factor has been approved for the prevention of severe mucositis among patients undergoing bone marrow transplant/HSCT<sup>194</sup> and should be considered in highly mucotoxic regimens such as those including total-body irradiation. Iron chelation should be considered for patients with abnormal liver function and increased iron overload.<sup>73</sup>

#### 6. Optimize antifungal therapy

##### 6.1 Select appropriate antifungal agent

Selecting the appropriate antifungal agent relies on evaluation of several factors including host, pathogen and drug spectrum and efficacy, site of infection, convenience, and cost (Table 20-12).

##### 6.2 Provide adequate duration of antifungal therapy (Table 20-13)

The key determinant of the duration of prophylaxis and therapy is the net state of immunosuppression. In neutropenic patients, prophylaxis can be stopped with resolution of myelosuppression. Among allogeneic HSCT recipients, *Candida* prophylaxis is usually discontinued at engraftment, although some continue for up to 100 days to provide protection during the period of risk for acute GvHD.<sup>195</sup> There are no data on when to stop prophylaxis among severely immunosuppressed cancer patients with normal neutrophil count. Extrapolation from the experience in HIV-positive patients suggests that stopping prophylaxis against *Pneumocystis jirovecii*, *Candida* spp., and *Cryptococcus neoformans* is safe once CD4+ count is  $\geq 200$  cells/ $\mu$ l for at least 3 months.<sup>196</sup>

Evidence-based guidelines for the duration of antifungal therapy for severely immunosuppressed patients are not available. A conservative strategy consists of treating an infection until complete resolution of all infection-related findings plus an additional period the duration of which depends on the likelihood and consequences of infection relapse (both of which depend on the net state of immunosuppression).

**Table 20-12** Practical considerations for the selection of antifungal agents in cancer patients**Host factors**

- Type of therapy: standard chemotherapy or allogeneic hematopoietic stem cell transplant (HSCT) (avoid amphotericin B (AmB) in the latter)
- **Immunosuppression: type, severity, and duration**
- **Hemodynamic stability, extent of infection:**
  - Patient with candidemia, hemodynamically stable and without organ involvement: use fluconazole. Use echinocandin or AmB if unstable or organ infection present
- **Prolonged exposure to antifungal agent and/or class.**
- **Organ dysfunction:**
  - Gut: select IV agents
  - Kidneys: select azoles or echinocandins
  - Liver: select AmB or echinocandins
- **Drug–food interaction: select IV agents**
- **Drug–drug interaction: avoid mould-active azoles if potential interaction is life-threatening**
- **Non-compliance with therapy: select IV agents**

**Pathogen and drug spectrum and efficacy**

Select agent likely to be effective against pathogen.

- *Candida* spp: avoid azoles in patients who develop candidemia while receiving fluconazole. Select an echinocandin instead with AmB product as alternative
- *Aspergillus* spp: mould-active azole or an AmB product
- Agents of zygomycosis: AmB product
- *Scedosporium apiospermum*: voriconazole, other azoles

**Site of infection and known tissue distribution of antifungal agent**

- **Ocular or central nervous system infection:**
  - If planning on using AmB select liposomal AmB (best tissue penetration)
  - If planning on using an azole, select fluconazole or voriconazole (best tissue penetration)
  - Avoid echinocandins (poor tissue penetration)
- **Urinary tract infection (pyelonephritis, cystitis, or other):**
  - Select fluconazole with or without 5-flucytosine

**Convenience and cost**

- Oral agent always preferable to IV products (unless impediments to absorption or compliance are present)
- Among the IV products, select the agent with the longest interval between doses

**Keep in mind the possibility of drug resistance**

Drug resistance may develop after prolonged exposure to antifungal agents, but is unlikely following a typical prophylactic and/or therapeutic course. For patients who develop azole-resistant IFI, use AmB or its lipid formulations and the echinocandins. The lack of response to antifungal therapy is most commonly due to clinical resistance related to host and/or drug delivery factors rather than microbial resistance.

### 6.3 Manage the interaction of antifungal agents with immunosuppressive and antineoplastic drugs

Two types of drug interactions must be considered when administering antifungal drugs and other agents to cancer patients<sup>197</sup> (Table 20-14).

- *Agents that downregulate the metabolism of commonly used immunosuppressants and anticancer drugs.* Several antineoplastic agents (busulfan, cyclophosphamide, other) and immunosuppressants used in allogeneic HSCT (corticosteroids, cyclosporin, tacrolimus and sirolimus) are metabolized through cytochrome P450 which is inhibited

by the antifungal azoles. Co-administration of azoles with these agents results in significant increases in drug exposure, with concomitant toxicity and overimmunosuppression. When the conditioning regimen includes busulfan and/or cyclophosphamide, the mould-active triazoles should be stopped at least 30 hours prior to starting these antineoplastic agents and can be resumed after five half-lives of the antineoplastic agent. The macrolide antimicrobials (erythromycin, clarithromycin, azithromycin) similarly increase the levels of immunosuppressants.

- *Agents that upregulate the metabolism of immunosuppressants and antifungal azoles.* These agents will cause

**Table 20-13** Indication for and duration of antifungal prophylaxis

Underlying condition	Yeasts		Moulds	
	During aplasia	After recovery♦	During aplasia	After recovery♦
AML/MDS induction/reinduction	++		±	±
ALL induction/reinduction	+		±	±
Autologous stem cell transplant	+			
Allogeneic stem cell transplant				
Non-myeloablative	+	+	±	±
Myeloablative	++	+	±	±
Matched related	+	+	±	±
Mismatched related	++	+	±	±
Matched unrelated	++	+	±	±

+, recommend; ++, strongly recommend  
 AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome.  
 ♦After marrow recovery or post-engraftment.  
 ± Preemptive therapy preferable if incidence <10% and diagnostic tools available on site with prompt turn-around time for results. Prophylaxis preferable if incidence >10% and/or optimal testing tools not locally available. Incidence does not relate to the entire population only but also to patient subsets; for example, among allogeneic stem cell transplant recipients, the risk is much higher if graft versus host disease (GvHD) is severe (acute II–IV or chronic, extensive).  
 Duration of prophylaxis:  
 1. GvHD: start on day 1 of immunosuppressive GvHD therapy until its end and CD4+ T cells ≥200/μL.  
 2. Other, non-allogeneic transplant: start on day 1 of chemotherapy and stop when absolute neutrophil counts ≥1000/μL.  
 3. Other, allogeneic transplant: same. One study suggests continuing until day +75  
 4. All conditions: avoid overlap between extended-spectrum triazoles (itraconazole, voriconazole, posaconazole) and certain chemotherapeutic agents (cyclophosphamide, busulfan) which may interact with the antifungal triazoles and cause toxicity.

subtherapeutic drug exposure with serious consequences for prevention and treatment of GvHD and management of IFIs.

**Synergistic nephrotoxicity:** Amphotericin B (AmB). Nephrotoxicity is one of the key mechanisms by which pharmacodynamic drug interactions occur with AmB.<sup>198</sup> Three types of interactions may result from AmB nephrotoxicity.

- Synergistic nephrotoxicity such as with cyclosporin and tacrolimus, cisplatin, and antimicrobials (aminoglycosides, high-dose trimethoprim-sulfamethoxazole, foscarnet and cidofovir). It is noteworthy that this synergistic toxicity may be precipitated by even a single dose of these agents, particularly when they are added in patients receiving calcineurin inhibitors.
- Delayed clearance of other renally excreted drugs as a result of AmB nephrotoxicity, hence causing accumulation of these agents with potential toxicity (melphalan, other antineoplastic agents, ganciclovir, 5FC).
- Electrolyte abnormalities that may either increase the toxicity of concomitant agents (hypokalemia in patients receiving digoxin) or may be worsened by co-administration of agents known to cause electrolyte abnormalities (foscarnet).

**Synergistic cardiotoxicity: Azoles** The increased risk of sudden death as a result of QT interval prolongation and torsades de

pointes is unlikely to develop with single-agent azole (or fluoroquinolone) therapy but rather as a result of a combination of factors such as in a cancer patient with cardiomyopathy with myeloma-related deposition disease, cardiotoxic antineoplastic therapy (anthracyclines, high-dose cyclophosphamide, others) who is also receiving prophylactic antifungal azole and a fluoroquinolone. The risk is further increased if azole drug interactions that lead to QT interval prolongation occur (e.g., receipt of quinidine or the antihistamines, astemizole or terfenadine).

Particular attention to the risk of this preventable yet potentially fatal complication is warranted.

**Other potential toxicities** The IV formulations of itraconazole and voriconazole are based on cyclodextrin solvents which accumulate in renal insufficiency (CrCl <30–50 mL/min). The potential toxicities from this accumulation in humans are not clear.

**6.4 Deploy a risk-based antifungal strategy.** A diagnostic-based preemptive or preventive strategy is preferable, in general, to treating established fungal infections, for several reasons listed earlier (under Diagnosis, General Principles), including the high risk for IFI and their serious consequences and the commonly delayed diagnosis in cancer patients.

The selection of the antifungal strategy should therefore be based on the risk of IFI in a given patient and the consequences of such IFI if early and appropriate therapy is not



**Table 20-14** Clinically significant interactions with azole antifungals in cancer patients.

Drug	Comments and recommended actions
<b>Azole affects drug: monitor for clinical toxicity and for toxic-range blood levels. Adjust dose</b>	
Calcineurin inhibitors: cyclosporin, tacrolimus, sirolimus	When starting azole, ↓ calcineurin inhibitors dose by 60–90% Closely monitor calcineurin inhibitors blood levels Increase dose when discontinuing azoles Voriconazole contraindicated with sirolimus
Corticosteroids: methylprednisolone, dexametasone, prednisolone	Monitor for toxicity
Antineoplastic agents: all-trans retinoic acid (ATRA), busulfan, ifosfamide, cyclophosphamide, docetaxel, paclitaxel, etoposide, irinotecan, vincristine, vinblastine	Avoid concurrent administration of azoles with these antineoplastic agents Use alternative non-azole antifungals May use azoles several days after last dose of antineoplastics
Benzodiazepines: midazolam, triazolam, alprazolam	Avoid all three agents with itraconazole. Use with caution with fluconazole, voriconazole, posaconazole. Frequent monitoring for toxicity. Use alternative agents: diazepam, bromazepam, estazolam, oxazepam and temazepam
Non-benzodiazepine hypnotosedatives/anxiolytics	Avoid buspirone with itraconazole and use with caution with fluconazole
Antidepressants/antipsychotics	With itraconazole: avoid pimozide, haloperidol, risperidone; caution with chlomipramine, nefazadone, trazodone. Use clozapine as alternative. With fluconazole: caution with amitryptiline, chlomipramine, fluoxetine. With voriconazole: avoid pimozide
Antiepileptics: phenytoin, phenobarbital, carbamazepine	With itraconazole: avoid phenytoin; caution with phenobarbital, carbamazepine With fluconazole: use phenytoin with caution With voriconazole: avoid phenobarbital, carbamazepine. Caution with phenytoin With posaconazole: caution with phenytoin Monitor phenytoin levels
Analgesics	Avoid alfentanil with fluconazole and itraconazole. Use fentanyl, sufentanil
Warfarin	↑ INR and risk of bleeding. ↓ warfarin dose, closely monitor INR
Calcium channel blockers	Monitor blood pressure and pulse. May need to ↓ dose of agent
Oral hypoglycemic agents	Avoid glipizide, glyburide with fluconazole and sulfonylureas with voriconazole Monitor serum glucose level and clinical signs of hypoglycemia with all agents
Statins: lovastatin, simvastatin, atorvastatin	Use of these agents with azoles is contraindicated Alternative suitable agents include fluvastatin and pravastatin Monitor for increased muscle pain, weakness, and rhabdomyolysis.

**Table 20-14** Clinically significant interactions with azole antifungals in cancer patients—cont'd

Drug	Comments and recommended actions
Antiarrhythmics: digoxin, quinidine, amiodarone, dofetilide	Avoid dofetilide. Avoid quinidine (↑QT interval and torsades de pointes). ↓ Digoxin dose by 60–75% with itraconazole. Monitor digoxin level and toxicity Caution with amiodarone and itraconazole
Antihistamines: astemizole, terfenadine	Avoid these agents with all azoles (↑QT interval and torsades de pointes).
<b>Drug affects azole</b>	
↓ Azole exposure	Monitor antifungal response. May need to ↑azole dose
Phenytoin, rifampin, rifabutin, isoniazid	Avoid with azoles. May use phenytoin with voriconazole but ↑ voriconazole by 25%. Monitor phenytoin concentration
↑ Azole exposure	
Protease inhibitors (PI)	↓ PI dose. Monitor for voriconazole toxicity
<b>Variable effects</b>	
Non-nucleoside reverse transcriptase inhibitors (NNRT)	NNRTIs may ↓ or ↑ azole level . Monitor for toxicity or lack of response Azole may ↑ NNRTI level. Monitor for toxicity
Also see Chapter 7. Experience with posaconazole is relatively limited and additional drug–drug interactions are likely. “Avoid”, interaction confirmed by controlled study, generally considered clinically significant, avoid combination if possible, or monitor closely. “Use Caution”, interaction noted in case report or can theoretically occur, generally not considered clinically significant, use combination cautiously. INR: International Normalized Ratio	

used. This risk-based strategy requires a balance between the need to protect the patient from a serious IFI on the one hand and avoiding toxicity, additional costs and risk for emergence of drug resistance on the other, if prophylaxis is given to a large number of patients who may not benefit from it. The risk stratification approach consists of three steps.

1. Identify the patient's risk category as high, intermediate or low, on the basis of the host and environmental exposure factors, and determine the period at risk (see [Tables 20-2 to 20-5](#)).
2. Monitor for infection according to the risk stratification above (intensive surveillance with serodiagnostic tools<sup>135</sup> and CT scans in the highest risk group versus minimal or no surveillance in the group at lowest risk for IFI).
3. Manage according to risk category for IFI. The risk-adjusted management strategies described in [Table 20-15](#) include the following: prophylaxis (primary or secondary (global, population and/or pathogen targeted)), empiric therapy, diagnostic-based preemptive therapy or treatment of established fungal infection.

#### 6.4.1 Primary prophylaxis

Primary prophylaxis of IFI in cancer patients has been studied mostly in neutropenic patients, and in HSCT recipients. Primary prophylaxis is best applied to patients at high risk for IFI and in whom the consequences of IFI are severe, even with proper therapy.<sup>199-202</sup>

#### Prophylaxis for yeasts

Fluconazole,<sup>13,195,203,204</sup> itraconazole oral solution (but not capsules),<sup>32,33,37,205-207</sup> voriconazole<sup>28,35</sup> and posaconazole effectively prevent the occurrence of invasive candidiasis in neutropenic patients undergoing induction remission for AML or myeloablative HSCT, but itraconazole is less well tolerated than the other azoles.<sup>206</sup> Micafungin is as effective as fluconazole and is well tolerated.<sup>31</sup>

#### Prophylaxis for yeasts and moulds

In addition to preventing candidiasis, micafungin prophylaxis was associated with a trend suggesting ability to prevent aspergillosis.<sup>31</sup>

Posaconazole was tested in patients with AML or myelodysplasia receiving induction therapy, and shown to be as effective as fluconazole, with good tolerability.<sup>37</sup> This study also showed that posaconazole was effective in preventing aspergillosis, with a reduction in fungal-related mortality. Posaconazole also was shown to prevent the occurrence of IPA in allogeneic HSCT recipients with GvHD in a randomized trial comparing with fluconazole.<sup>36</sup>

The use of itraconazole oral solution in HSCT recipients also resulted in a reduction in the frequency of IPA in two randomized trials but as in the trials in AML, about 25% of patients discontinued itraconazole because of gastrointestinal side effects.<sup>30,207</sup> A recent meta-analysis of itraconazole trials suggested that there is a reduction in *Aspergillus* infections but only if a certain threshold of bioavailable dosing

**Table 20-15** Antifungal strategies: definitions, goals, risk categories and clinical examples

Antifungal strategy	Goal	Start of therapy	Colonization or infection	Patient risk for serious fungal infections	Examples in allogeneic HSCT recipients
<b>Prophylaxis</b>					
<i>Primary</i>	Reduce rate of infection	At initiation of immunosuppression	Absent	Patients at high risk for serious infection	
Global	Same	Same	Same	All patients	Yeast and mould-active agent to all recipients
Population targeted	Same	Same	Same	Subgroup at high risk for morbidity from infection with any fungal pathogen	Same as above but limited to patients with severe GvHD♦
Pathogen targeted	Same	Same	Same	Subgroup at greatest risk for morbidity from infection with certain pathogens	Yeast prophylaxis to recipients not at serious risk for mould infection
<i>Secondary</i>	Prevent recurrence of infection	Same or chronic suppressive therapy if ongoing immunosuppression	Same	Subgroup with a controlled prior infection but additional anticancer therapy planned	Patients whose aspergillosis was controlled but develop severe GvHD♦ requiring immunosuppression
<b>Therapy</b>					
Empiric	Treat possible infection at early stage	Persistent fever or non-specific findings	Absent	Intermediate risk: patients in whom the risk for infection is not high enough to warrant prophylaxis	Initiation of antifungal therapy in all allogeneic HSCT recipients who develop persistent fever or non-specific findings
Diagnosis-based Preemptive	Suppress infection at early stage	At first clinical or laboratory evidence <sup>†</sup> that connotes increased risk of infection with a significant pathogen	Present but patient may be asymptomatic	Patients in whom the risk for infection is not high enough to warrant prophylaxis	Initiation of mould-active agent in allogeneic HSCT recipients whose serial testing connotes an increased risk for a serious infection <sup>†</sup>



**Table 20-15** Antifungal strategies: definitions, goals, risk categories and clinical examples—cont'd

Antifungal strategy	Goal	Start of therapy	Colonization or infection	Patient risk for serious fungal infections	Examples in allogeneic HSCT recipients
Therapy of established infection	Treat established infection	At diagnosis of infection	Present	Low risk: patients in whom the risk for serious consequences from infection is too low to warrant prophylaxis, empiric or preemptive therapy	Patient who develops infection while in remission and off all immunosuppression.
<p>HSCT: Hematopoietic stem cell transplant</p> <p>♦ Severe GvHD, graft vs host disease; relies on established parameter of host susceptibility to determine the group at risk (e.g., <math>\geq 1</math> mg/kg/day of prednisone for &gt;10 days).</p> <p>† Laboratory evidence: in a patient at risk: (a) positive serum <i>Aspergillus</i> galactomannan antigen test or <math>\beta</math>-D-glucan assay; (b) positive culture for significant pathogens such as <i>Aspergillus</i> spp.; (c) halo sign, cavitation or crescent formation on CT scan of chest in a patient at risk.</p>					

is used.<sup>205</sup> Its ability to prevent IFI has been associated with trough itraconazole concentrations >500 ng/ml measured by high-pressure liquid chromatography (HPLC), which is best achieved with the IV formulation (followed by the oral solution if the gastrointestinal function is intact). The oral capsule formulation suffers from erratic bioavailability and is best avoided.

Taken together, the results of these studies using mould-active azoles and their impact in reducing the incidence of IPA are appealing.<sup>208</sup> However, these findings should be balanced against our significantly improved ability for the early detection of fungal infections and the potential undesirable consequences including toxicities, drug–drug interactions, costs, and emergence of resistance.<sup>209</sup> Finally, it is important to keep in mind the limitations of global prophylaxis, defined as prophylaxis for all patients with a certain diagnosis (e.g., all patients undergoing therapy for acute leukemia). Indeed, the risk among these patients is variable. It is highest among patients with relapsed/refractory disease who should probably receive primary yeast and mould prophylaxis; the risk is somewhat lower among patients undergoing remission induction chemotherapy in whom yeast prophylaxis and a surveillance guided diagnostic-based preemptive strategy is best. The lowest risk for IFI is during consolidation therapy. These patients may not even require systemic prophylaxis (see Table 20-13).

Because of these potential consequences, several factors should be taken into consideration in determining if prophylaxis is appropriate at a specific treatment center, for a given patient or patient population to target a specific infection (depending on their risk for IFI as assessed above) or if prophylaxis should be withheld and a diagnostic-based preemptive strategy used instead. For example, a center with a significant incidence of IFIs among its cancer and HSCT patients may provide anticandidal prophylaxis with fluconazole while at the same time choosing a diagnostic-based preemptive strategy to

target IMIs (provided the treatment facility has timely access to the results of serodiagnostic markers and CT scan). On the other hand, prophylaxis may not be needed at centers in which the incidence of candidiasis and aspergillosis is very low.<sup>205</sup>

A randomized trial in patients with prolonged neutropenia showed no benefit for nebulized AmB.<sup>210</sup> Preliminary reports suggest that aerosolized lipid formulations of AmB are safe and may be effective.<sup>211-213</sup>

#### 6.4.2 Secondary prophylaxis

Because the risk of reactivation of IMIs is high following resumption of immunosuppression, secondary prophylaxis is indicated in such patients.<sup>214</sup>

A recent review of secondary antifungal prophylaxis included 197 patients with previous proven or possible IA who received additional cytotoxic chemotherapy or HSCT while receiving secondary prophylaxis with amphotericin B, itraconazole or flucytosine, or combinations of these agents. Documented relapse of IA was only 16% (31 of 197) of those who received prophylaxis compared to 62% (26 of 42) of those who did not ( $P < 0.0001$ ).<sup>215</sup> Additional experience has since confirmed the importance of secondary prophylaxis.<sup>216,217</sup> In allogeneic HSCT patients with history of aspergillosis, the risk of reactivation was lower if treatment was given for >30 days and radiographic abnormalities resolved.<sup>218</sup> Other risk factors for reactivation include the status of the underlying disease, the type of conditioning regimen, the duration of neutropenia and the presence of risk modifiers such as CMV disease and severe GvHD.

Options for secondary prophylaxis include AmB and its lipid formulations, caspofungin, itraconazole, voriconazole and lipid AmB followed by voriconazole.<sup>215-217</sup>

Because of the risk of late infection relapse, long-term suppressive antifungal therapy should also be given to patients who survived an episode of IPA related to an illness associated with permanent immunosuppression.

In addition to secondary chemoprophylaxis, strategies to abbreviate the duration of neutropenia, such as the use of reduced-intensity conditioning regimens and peripheral blood stem cells, and the use of granulocyte transfusions may be employed.<sup>191,193</sup>

#### 6.4.3 Empiric therapy

Empiric antifungal therapy has been the standard of care in the management of the febrile neutropenic cancer patient since the 1980s.<sup>219</sup> The strategy consists of starting an antifungal agent in neutropenic patients after 4–7 days of persistent fever without apparent cause, despite the use of appropriate antibacterial agents. Different drugs have been tested, including deoxycholate AmB (D-AmB),<sup>220</sup> fluconazole,<sup>221,222</sup> the lipid formulations of AmB,<sup>223–225</sup> itraconazole,<sup>226</sup> voriconazole,<sup>227</sup> and caspofungin.<sup>228</sup> Despite problems in study design<sup>229</sup> and concerns about cost and toxicity, empiric therapy remains the most frequently used strategy in neutropenic patients. However, if an antifungal agent is started on the basis of persistent fever as the sole criterion, a significant number of patients will receive antifungal therapy unnecessarily, particularly among recipients of fluconazole prophylaxis, because persistent fever in these patients is most commonly due to various factors, such as uncontrolled occult bacterial infection, viral infection, drug fever and others. Empiric therapy should be reserved to patients at intermediate risk for IFI, when serodiagnostic markers are not readily available and fluconazole prophylaxis is not given.

#### 6.4.4 Diagnostic-based preemptive therapy

This strategy relies on the administration of antifungal therapy to patients with early evidence of IFI<sup>208</sup> such as persistent fever plus any of the following: pleuritic chest pain, pleural rub, and/or positive serum galactomannan,  $\beta$ 1,3-d-glucan or PCR, or new and suggestive pulmonary infiltrates on chest CT scans.<sup>139</sup> Diagnostic-based preemptive therapy has been successfully tested in neutropenic patients<sup>125,142</sup> though no standard recommendations exist.<sup>140,230</sup> This strategy significantly decreases the risks associated with prophylaxis and empiric therapy as discussed above.

#### 6.4.5 Treatment of documented infection

Patients at low risk for IFI do not benefit from antifungal prophylaxis or diagnostic-based preemptive or empirical antifungal therapy. Rather, every effort should be made to document an IFI. These infections are rare in low-risk patients who are usually stable with adequate platelet counts allowing definitive diagnostic procedures.

The mainstay of antifungal treatment for IFI in patients with cancer was D-AmB until the recent availability of several new agents.

The drugs available for the treatment of IFI include D-AmB; the three lipid formulations of AmB: AmB in lipid complex (ABLC), in liposome (L-AmB), and in colloidal dispersion (ABCD); 5-flucytosine (5FC); the azoles fluconazole, itraconazole, voriconazole, and posaconazole; and the echinocandins caspofungin, micafungin, and anidulafungin (Table 20-16).

The selection of the antifungal agent must take several factors into consideration (see Table 20-12). For example, allogeneic HSCT recipients are at great risk for D-AmB related nephrotoxicity.<sup>198,231,232</sup> Therefore, this drug should be

avoided and a lipid AmB preparation used instead, because these drugs are at least as efficacious and much less nephrotoxic, and one formulation, L-AmB, is associated with significantly fewer infusion-related toxicities.

#### Invasive candidiasis

There are eight published randomized trials on the treatment of candidemia and invasive candidiasis.<sup>233–240</sup> While a reasonable proportion of patients in these trials had cancer as the underlying disease, these patients were not analyzed separately and the proportion of cancer patients who were neutropenic was low (~10%), making it difficult to select a “drug of choice.” Therefore, the choice of an appropriate drug for the treatment of candidemia or invasive candidiasis in neutropenic patients must be guided by other factors (see Table 20-12).

The first consideration is recent exposure to fluconazole which increases the likelihood of *C. glabrata* or *C. krusei* infection.<sup>241,242</sup> In this case, an echinocandin or a lipid AmB formulation would be more appropriate. Voriconazole is a viable option for *C. krusei* infections. Another consideration is whether the patient is hemodynamically stable or not, in which case an echinocandin would be preferable. For more details, see Table 20-12.

Catheter management in candidemia is controversial but in neutropenic patients, its removal is less likely to have an impact on the outcome, because the gut is the most likely source of infection.<sup>243</sup> For complete information on managing candidemia, please refer to Chapter 8.

#### Invasive aspergillosis

The IDSA guidelines recommend voriconazole as the drug of choice for primary treatment of invasive aspergillosis on the basis of a randomized trial which showed superior response and better survival compared with D-AmB.<sup>244,245</sup> An alternative option is liposomal AmB, at the dose of 3 mg/kg/day.<sup>246</sup> For salvage therapy, an AmB lipid formulation is preferred. Alternatives include itraconazole or posaconazole, and an echinocandin (caspofungin). For patients who develop voriconazole-refractory aspergillosis, considerations include a change of class to an AmB product or echinocandin (caspofungin) or a combination of agents. The combination of echinocandin with voriconazole has been evaluated in limited studies with variable results.<sup>247,248</sup> Randomized trials are warranted.

#### Other mycoses

For the treatment of zygomycosis, a lipid formulation of AmB is the drug of choice.<sup>107,249</sup> Posaconazole, an oral azole active against some agents of zygomycosis, may be considered as follow-up therapy to an AmB product if the species causing infection is known to be susceptible<sup>250,251</sup> (Table 20-17). Surgical treatment and reversal of immunosuppression and ketoacidosis are critical components of therapy.<sup>118,119</sup>

Fusariosis is probably best treated with a lipid formulation of AmB, although successes have been reported with voriconazole and posaconazole.<sup>252</sup> The latter agents should be considered if the species causing infection is known to be susceptible to these agents (see Table 20-17).

Table 20-18 suggests the optimal therapies for less common mycoses in cancer patients. It is important to keep in mind that more than one fungal pathogen may be causing infection, particularly in the setting of severe immunosuppression.<sup>21</sup>

**Table 20-16** Therapeutic options for the treatment of invasive fungal infections in cancer patients and in HSCT recipients

Disease	Drug	Dose, Route	Comments
Candidemia*	Caspofungin	70 mg d1 → 50 mg/d, IV	Cancer diagnosis comprised ~20–25% of patients in 8 randomized studies; neutropenia present in ~10% of patients (in some studies neutropenia was an exclusion criterion)
	Micafungin	100 mg/d, IV	
	Anidulafungin	200 mg → 100 mg/d, IV	
	L-AmB**	3 mg/kg/d, IV	
	Voriconazole	6 mg/kg 12/12 h d1 → 3 mg/kg 12/12 h, IV or 200 mg 12/12 h, PO	
	Fluconazole	800 mg d1 → 400 mg/d, IV → PO	
Aspergillosis	Voriconazole (first line)	6 mg/kg 12/12 h d1 → 4 mg/kg 12/12 h, IV or 300 mg 12/12 h PO	Salvage therapy: caspofungin 70 mg/d IV or posaconazole 200 mg 6/6 h or 400 mg 12/12 h PO
	L-AMB	3 mg/kg/d, IV	
Fusariosis	L-AMB	3 mg/kg/d, IV	Salvage therapy: voriconazole 6 mg/kg 12/12 h d1 → 4 mg/kg 12/12 h IV or 300 mg 12/12 h PO or posaconazole 200 mg 6/6 h or 400 mg 12/12 h PO
	ABLC	5 mg/kg/d, IV	
Zygomycosis	L-AMB	3 mg/kg/d, IV	Salvage therapy: posaconazole 200 mg 6/6 h or 400 mg 12/12 h PO
	ABLC	5 mg/kg/d, IV	

L-AMB, liposomal amphotericin B; ABLC, amphotericin B in lipid complex; amphotericin B colloidal dispersion is another option in aspergillosis, fusariosis and zygomycosis; dose 4–6 mg/kg/d, IV

\*Listed only drugs studied in randomized clinical trials;

\*\*deoxycholate amphotericin B is an option, but should be avoided because of toxicity.

## 7. Failure to respond to antifungal therapy

Despite all efforts, some patients may have clinical and/or radiologic deterioration suggesting progressive infection. It is critical in such a setting to rule out potentially modifiable factors that may improve response. These include:

- ensuring that an adequate dosage schedule of the appropriate antifungal drug is prescribed and if the agent is given orally, that the patient is compliant with therapy and does not suffer any condition that may impair adequate drug exposure (nausea, vomiting, diarrhea, gut GvHD, mucositis, other)
- ruling out the possibility that clinical and/or radiologic deterioration is due to IRIS (i.e., not failure of therapy)
- obtaining additional sampling to diagnose other concomitant infections (fungal and other), which may require a different therapeutic strategy
- searching for a focus of infection which may need drainage, removal of prosthetic device or other. A PET/CT can be particularly helpful in such a setting<sup>157,159</sup>
- considering the possibility that the clinical findings may no longer be due to ongoing infection but to its residual effects, such as infarcts in patients with angioinvasive mould infection (e.g., CNS infarct with persistent neurologic deficits)
- reevaluating the net state of immunosuppression which may be the key reason for failure to respond. In such case, decreasing immunosuppression and enhancing

immunity may be the most promising strategies to improve outcome.

## *Pneumocystis jiroveci*

Risk groups in which prophylaxis should be considered against *P. jiroveci* include lymphoreticular cancers and myeloablative therapy with HSCT in which treatment regimens result in suppression of T lymphocyte immunity (e.g., corticosteroids, purine analogs, monoclonal antibodies directed against T cells and others). Infection can be prevented by trimethoprim-sulfamethoxazole (TMP-SMX) which can be given as one double-strength tablet (160 mg TMP plus 800 mg SMX) once or twice daily, but three times weekly also is effective. Aerosolized pentamidine given once monthly at a 300 mg dose is an alternative, avoiding some of TMP-SMX's toxicities, but is less effective. Dapsone and atovaquone are also alternatives. For more details, please refer to Chapter 17.

## Prognosis of fungal infections in cancer patients

Invasive fungal infections represent a major complication in cancer patients and in HSCT recipients because they are associated with high death rates (30–50% in candidemia,<sup>253-257</sup> 60–80% in aspergillosis,<sup>258,259</sup> and >80% in zygomycosis and fusariosis).<sup>19,106,260</sup> This poor outcome is the result of the major imbalance between the weakened host and the offending



**Table 20-17** Spectrum of activity of currently available antifungal agents

	Amphotericin B	Fluconazole	Itraconazole	Voriconazole	Posaconazole	5FC	Echinocandins♦
<i>MOULDS</i>							
<i>Aspergillus</i> spp.							
<i>A. fumigatus</i>	++++ ‡	–	++++	++++ ‡	++++	–	++++
<i>A. flavus/terreus</i>	++	–	++++	++++ ‡	++++	–	++++
<i>A. niger</i>	++++ ‡	–	++++	++++ ‡	++++	–	++++
<i>A. lentulus</i>	–	–	–	–	–	–	–
<i>Zygomycetes</i> †	++++ ‡	–	++	–	+++	–	–
<i>Fusarium</i> species†	+++ ‡	–	++	++ †	++ †	–	–
<i>Trichoderma</i>	+++	?	+++	?	?	–	–
<i>Penicillium marneffei</i>	++++ ‡	–	+++♠	+++	+++	–	–
<i>Scedosporium</i>							
<i>S. apiospermum</i>	++	++	+++	+++‡	+++	–	+++
<i>S. prolificans</i> **	–	–	–	–	–	–	–
<i>Paecilomyces</i>							
<i>P. lilacinus</i>	+	–	+++	++++	++++	–	+++
<i>P. variotii</i>	++++ ‡	–	++++	++++	++++	–	+++
<i>Acremonium</i> spp.	++++ ‡	–	+++♠	+++	+++	–	–
<i>Scopulariopsis brevicaulis</i>	++ ‡	–	–	++	–	–	?
Dematiaceous moulds **	+++	–	++++‡	++++	++++	variable	?

YEASTS							
<i>Trichosporon</i>	++	++++ ‡	++++	++++	++++	–	–
<i>Blastoschizomyces capitatus</i>	+++	++++ ‡	++++	++++	++++	++	–
<i>Saccharomyces</i>	++++	–	++++	++++	++++	++++	+++?
<i>Rhodotorula</i>	++++ ‡	+++	+++	+++	+++	++++	–
<i>Malassezia</i>	+++	++++	++++	++++ ‡	+++	–	–
<i>Pichia</i>	++++ ‡	+++	+++	+++	+++	+++	+++?
<i>Cryptococcus</i> species	++++ ‡	++++ ♠	+++	+++	+++	++++	–
<i>Candida</i> spp.							
<i>C. albicans</i>	++++	++++ ‡	++++	++++	++++	++++	++++
<i>C. glabrata</i>	++++	++	+++	+++	++++	++++	++++ ‡
<i>C. krusei</i>	++++	–	++	+++	++++	++++	++++ ‡
<i>C. parapsilosis</i>	++++	++++ ‡	++++	++++	++++	++++	++
<i>C. lusitaniae</i>	+++	++++	++++	++++	++++	++++	++++
<i>C. tropicalis</i>	++++	++++ ‡	++++	++++	++++	++++	++++
<p>?, conflicting data.  ++++ &gt;90% susceptible; +++ 50–90% susceptible; ++ &lt;30% susceptible; (–) &lt;5% susceptible.  ‡Drug of choice in severe infections.  ♠Drug of choice in moderately severe infection, as alternative agent or follow-up to drug of choice.  †Species-dependent susceptibility: <i>Rhizopus</i> spp. and <i>Mucor</i> spp. are only consistently susceptible to amphotericin B, while <i>Rhizomucor</i> spp., <i>Absidia</i> spp. and <i>Cunninghamella</i> spp. are also susceptible to itraconazole and posaconazole. <i>Fusarium solani</i> and <i>F. verticillioides</i> are resistant to azoles, and exhibit higher MICs for amphotericin B than other <i>Fusarium</i> spp. <i>F. oxysporum</i> and <i>F. moniliforme</i> may be susceptible to voriconazole and posaconazole.  Note: 5-Flucytosine is active against <i>Cryptococcus neoformans</i> and certain <i>Candida</i> species, including <i>C. albicans</i> and <i>C. glabrata</i>. <i>Malassezia furfur</i> and <i>M. pachidermitis</i> are susceptible to azoles but resistant to 5-FC; variable susceptibility of <i>M. pachidermitis</i> to amphotericin B.  ◆Echinocandins: caspofungin, micafungin, anidulafungin.  **Dematiaceous moulds: Phaeohyphomycosis (pigmented cell wall); includes several species (see text).</p>							

**Table 20-18** Diagnostic and therapeutic considerations in the management of less common fungal infections in cancer patients

Pathogen	Clinical manifestations	Characteristics	Treatment
<b>MOULD</b>			
<i>Scedosporium apiospermum</i>	Pneumonia, sinusitis, CNS infection, disseminated	Hyaline mould	Voriconazole drug of choice.
<i>Scedosporium prolificans</i>	Cellulitis, osteomyelitis, disseminated. Blood cultures may be positive	Dematiaceous mould Aleuroconidia may be present	Resistant to all antifungal agents
<i>Paecilomyces lilacinus</i>	Pneumonia, peritonitis in CAPD, osteomyelitis, and prosthetic valve endocarditis. Blood cultures may be positive	Hyaline mould Aleuroconidia may be present	Newer azoles. Not susceptible to AmB
<i>Paecilomyces variotii</i>	Pneumonia, peritonitis in CAPD, osteomyelitis, and prosthetic valve endocarditis. Blood cultures may be positive	Hyaline mould Aleuroconidia may be present	AmB drug of choice. Newer azoles as alternative
<i>Acremonium</i> spp	Endophthalmitis, keratitis, disseminated. Blood cultures may be positive	Hyaline mould. Aleuroconidia may be present	Voriconazole, surgery
<i>Trichoderma</i> spp.	Peritonitis in CAPD, localized, disseminated. Blood cultures may be positive	Hyaline mould  Aleuroconidia may be present	Catheter removal  Optimal antifungal therapy remains unclear
Dematiaceous moulds**	CNS infection (mass effect), sinusitis, disseminated	Fontana-Masson stain reveals cell wall pigmentation of hyphae. Pseudohyphae and yeast forms may also be seen. Serum $\beta$ DG test and serum <i>Aspergillus</i> GM may be positive with some species (Table 20-11)	Surgery plus itraconazole or newer triazoles. AmB+5-FC may also be effective. Poor outcome when disseminated
<b>DIMORPHIC</b>			
<i>Penicillium marneffei</i>	Limited to Southeast Asia and residents of southern China. Systemic illness with skin lesions, cough, lymphadenopathy and weight loss. Similar to the manifestations of histoplasmosis in HIV-infected individuals	Yeast forms without budding at 37 °C; hyphae at 25°C. Unlike <i>Histoplasma</i> which divide by budding, <i>P. marneffei</i> divides by fission	AmB is drug of choice in severe infections but relapse is common once stopped. Itraconazole is drug of choice in moderately severe infection, or as a follow-up to 2 weeks of AmB



**Table 20-18** Diagnostic and therapeutic considerations in the management of less common fungal infections in cancer patients—cont'd

Pathogen	Clinical manifestations	Characteristics	Treatment
<b>YEAST</b>			
<i>Trichosporon asahii</i> , <i>inkii</i> , <i>mucooides</i>	Similar to acute, chronic disseminated candidiasis	True hyphae, pseudohyphae, arthroconidia and blastoconidia. Cross-reacts with <i>C. neoformans</i> antigen test. Positive serum $\beta$ DG test	Fluconazole, other triazoles.
<i>Blastoschizomyces capitatus</i>	Similar to acute, chronic disseminated candidiasis though organ involvement more common than with candidiasis. Commonly in neutropenic patients with acute leukemia. Most observed in Europe, esp. Italy, Spain, France	True hyphae, pseudohyphae, arthroconidia and blastoconidia. Cross-reacts with <i>Aspergillus</i> galactomannan test. Positive serum $\beta$ DG test	Fluconazole, other triazoles, high-dose AmB. May have decreased susceptibility to AmB, triazoles, 5FC. Resistant to echinocandins
<i>Malassezia furfur</i>	CVC-related infection, especially if receiving lipids. Distinguish superficial folliculitis from hematogenously spread skin lesions	Yeast on Gram stain of blood culture but growth requires lipid supplementation	Remove CVC. AmB, Fluconazole, other triazoles. 5-FC not effective. Infection control measures
<i>Malassezia pachydermatis</i>	Fungemia. Nosocomial outbreaks noted	Growth does not require lipid supplementation	Fluconazole, voriconazole. Variable susceptibility to AMB. 5FC not effective.
<i>Pichia anomala</i> , <i>ohmeri</i>	Outbreaks with nosocomial transmission ( <i>P. anomala</i> ). Fungemia, pneumonia, endocarditis, ventriculitis, other	Colony similar to <i>Candida</i> species. Ascoconidia production by <i>Pichia</i> differentiates it from <i>Candida</i> species	Remove CVC. AmB, fluconazole + 5FC. Newer azoles, echinocandins may be effective. Infection control measures
<i>Saccharomyces cerevisiae</i>	Fungemia. Similar to acute disseminated candidiasis with ocular involvement. <i>S. cerevisiae</i> subtype <i>boulardii</i> fungemia is common and usually follows probiotic administration. Nosocomial transmission possible.	Positive serum $\beta$ DG test	AmB, 5FC. Fluconazole not effective. Newer azoles, echinocandins may be effective
<i>Rhodotorula rubra</i> , <i>glutinis</i>	Fungemia. Rarely, endocarditis, peritonitis, meningitis, endophthalmitis, disseminated disease.	Produces carotenoid pigments ranging from a yellowish to red. Frequently associated with water.	AmB+/-5FC. CVC removal. Resistant to echinocandins
<p>AmB, amphotericin B; <i>C. neoformans</i>, <i>Cryptococcus neoformans</i>; CNS, central nervous system; CVC, central venous catheter; 5FC, 5-flucytosine; CAPD, continuous ambulatory peritoneal dialysis; <math>\beta</math>DG, <math>\beta</math>1-3-d-glucan test; <i>Aspergillus</i> GM, serum <i>Aspergillus</i> galactomannan antigen test.</p> <p>◆ Echinocandins: caspofungin, micafungin, anidulafungin.</p> <p>**Dematiaceous moulds: phaeohyphomycosis (pigmented cell wall); includes several species (see text and Table 20-8). <i>Cladophialophora bantiana</i> is particularly neurotropic and the most common cause of cerebral phaeohyphomycosis though it is very rare in cancer patients.</p>			

fungus. Indeed, host factors are by far the most important prognostic factors for any IFI in these patients and reflect, in most cases, the net state of immunosuppression and the presence of co-morbidities. In neutropenic cancer patients, persistence of neutropenia in the setting of an IFI is almost universally fatal.<sup>261</sup> Likewise, in candidemic patients, severity of illness scores strongly influence outcome.<sup>255,262</sup> In HSCT recipients, factors related to severe T cell immunosuppression, including severe GvHD and the use of high-dose corticosteroids, are the most important prognostic factors.<sup>258,259,263,264</sup>

Reversing the imbalances that favor pathogen over host should always remain the focus of every effort aimed at preventing and managing IFIs in cancer patients.

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# Fungal infection in the organ transplant recipient

Robert H. Rubin

The remarkable success being achieved by organ transplantation today (e.g., >90% 1-year patient and graft survival following cadaveric kidney transplantation) has changed the approach to the care of patients with chronic, progressive organ failure. A few decades ago, transplantation was considered a last desperate therapy, marred by technical complications, systemic infection, rejection, and a broad array of complications of immunosuppression, particularly corticosteroids. Today, we recognize organ transplantation as the most effective means of rehabilitating patients with organ failure of diverse etiologies. This is not to say that all the problems have been solved; rather, an approach has been developed that increases the chances of success. Important principles have been developed that serve as a foundation for clinical efforts.<sup>1-3</sup>

Effective antimicrobial therapy in the transplant recipient is particularly challenging for a number of reasons.

1. Microbial load is a major determinant of the prognosis for a given patient with infection. The impaired inflammatory response to microbial invasion that is engendered by immunosuppressive therapy commonly results in an occult presentation: signs and symptoms are attenuated until late in the infection; radiologic findings are often more subtle; dermatologic manifestations are dampened so that classic findings are absent and skin lesions are often non-specific. The microbial load is invariably greater in the transplant patient than in the normal host with a similar illness. The blandness of the response to many fungal infections only adds to diagnostic difficulty. Since prognosis is directly related to how quickly the appropriate diagnosis is made and effective therapy prescribed, an aggressive approach to evaluation of the patient is mandatory. CT scan rather than conventional radiography is the appropriate response to chest symptoms and/or minimal findings on conventional radiology; skin lesions as well as chest nodules require biopsy; and unexplained fevers require extensive evaluation.<sup>1,3-5</sup>
2. Antimicrobial therapy, particularly antifungal therapy, in the transplant recipient is likewise more complex than in other populations. There are three modes of therapy that need to be considered: prophylactic, therapeutic, and preemptive. Prophylactic therapy is administered to an entire population of uninfected patients when the risk of clinical disease is deemed great enough to justify the intervention. An example is the performance of a transplant in

the face of aerosolization of one of the endemic systemic mycoses. Antifungal prophylaxis provides protection against clinical disease. The therapeutic use of antifungal drugs is the treatment of invasive infection, often due to the failure of prophylactic regimens. Preemptive therapy is utilized when asymptomatic patients are regarded as being at increased risk of invasive infection on the basis of a laboratory or clinical observation. Antimicrobial therapy in transplant patients requires more prolonged therapy than in other patient populations because of the increased microbial burden and the continuing need for immunosuppression. As a result, toxicity is usually greater and antimicrobial resistance may be selected.<sup>1,3,5</sup>

3. The range of organisms to be considered is far broader than that causing similar clinical presentations in the normal host. In the transplant patient, not only are the usual bacteria (e.g., *Staphylococcus aureus*, a variety of streptococci, *E. coli* and other Gram-negative bacilli) and viruses (e.g., influenza, respiratory syncytial virus, and hepatitis B and C) of concern, but more unusual pathogens must be considered. These unusual pathogens include fungi, not only the traditional organisms such as *Candida albicans* but also opportunistic species such as *Aspergillus* and *Cryptococcus*. In addition, there is an increasing problem with what has been termed the “new and emerging” organisms (such fungi as *Scedosporium*, *Fusarium*, and the zygomycetes). Uncommon bacterial (*Nocardia*, *Mycobacterial*, and pathogenic corynebacterial) and viral infections (e.g., SARS and West Nile virus) add significantly to the management challenges faced by the clinician. On the one hand, the impact of traditional pathogens is greater in this patient population; on the other hand, the effects of newly introduced pathogens will be particularly threatening in transplant patients and other immunosuppressed hosts. They are truly “sentinels” for excess trafficking in potential pathogens in the environment.<sup>7</sup>
4. There is a direct link between early diagnosis and effective therapy. Indeed, the patient would be well served by considering diagnosis and therapy together as part of an infection management program, one that includes not only antimicrobial therapy but also diagnostics that inform the use of these drugs. Although culture techniques remain the cornerstone of disease management, new approaches such as antigen detection, polymerase chain reaction (PCR) detection of microbial nucleic acids, and newer radiologic

procedures hold great promise for early diagnosis and the deployment of effective therapy. In contrast, serologic diagnosis (the testing for specific antibodies in the serum), which is measuring the immunologic response to a particular antigen, is far less sensitive and is usually not part of the diagnostic evaluation in immunocompromised patients with active infection. The major application of serologic techniques is prior to transplant when these measurements can help assess the risk of particular infections. The key concept here is to forge the link between early diagnosis and the preventive and therapeutic use of antimicrobials.<sup>1,2</sup>

## Risk of fungal infection in the organ transplant recipient

The risk of fungal infection in the organ transplant recipient is determined by the interaction of four factors.

### Net state of immunosuppression

The net state of immunosuppression is a complex function that is made up of a number of factors; the level of immunosuppressive therapy being administered is the primary determinant but, in addition, other entities contribute to this function: neutropenia, protein calorie malnutrition (and, probably, other metabolic disturbances such as uremia and hyperglycemia); systemic infection with one or more of the immunomodulating viruses (cytomegalovirus (CMV), Epstein-Barr virus, human herpesvirus-6; hepatitis viruses B and C, and the human immunodeficiency virus); and other factors such as immune response genes. The importance of these additional contributors to the net state of immunosuppression is underlined by the following observations: transplant patients with a serum albumin level of <2.5 gm/dl have a 10-fold increase in the incidence of life-threatening infection; 90% of opportunistic infection occurs in the setting of viral infection. For those patients without viral infection, there is a high probability of an excessive environmental hazard being present to account for the invasive infection; that is, viral infection increases the net state of immunosuppression such that the individual is susceptible to invasive infection.<sup>1,2,4</sup>

### Environmental exposures

Excessive environmental exposures can occur within the community or in the hospital. In the community, aerosols can be created and inhaled during the course of construction, gardening or farm work. Hospital exposures of importance that can result in invasive fungal infection can be divided into two general categories: domiciliary and non-domiciliary. Domiciliary refers to the acquisition of infection on the ward where the patient is housed, with outbreaks of this type being marked by clustering of cases in time and space. Non-domiciliary exposures occur as a patient travels in the hospital for essential procedures. Exposures of this sort can occur in the halls of the hospital, the operating room, the radiology or bronchoscopy suites and elsewhere. Non-domiciliary outbreaks are not uncommon, with the clue of a possible hazard coming from the diagnosis of infection at a point in time when the net state of immunosuppression

should not have been great enough by itself for such infection to occur. Excessive amounts of infectious particles in the potable water and/or the air that is inhaled can produce invasive disease, particularly in the transplant situation in which the cell-mediated response to fungal tissue invasion is most affected by the immunosuppressive therapy being administered.<sup>5-7</sup>

In recent years a growing problem has been the person-to-person spread of azole-resistant *Candida* infection (both resistant *C. albicans* and non-*albicans Candida* strains being spread in this fashion), on the unwashed hands of medical personnel.<sup>1,5,7,10,11</sup>

The dimorphic fungi (*Blastomyces dermatitidis*, *Coccidioides immitis* and *Histoplasma capsulatum*) grow in the soil as a mould composed of a mesh of septate hyphae bearing conidia (the infectious particles) that can be aerosolized when the ground bearing these organisms is manipulated during urban renewal projects, "clean-up" efforts, and explorations of sites where the soil is "enriched" with bird and animal droppings (e.g., bat caves, chicken roosts, and beaver habitats). Inhalation of aerosolized conidia will initiate clinical infection in the lungs, with the tissue invasive form being yeast.<sup>1,2,8,9,12,13</sup>

The clinical consequences of tissue invasion by these organisms can be quite diverse. Primary infection occurs in the first month after exposure. This may be asymptomatic or be associated with a flu-like syndrome. In addition, hypersensitivity manifestations such as erythema multiforme, erythema nodosum, and reactive arthritis are common manifestations of primary infection and the development of immunity in a normal host. In transplant patients, such immune effects are uncommon, whereas progressive primary pneumonia and postprimary dissemination via the bloodstream are common. These dimorphic fungi have a similar epidemiology and pathogenesis: a pulmonary portal of entry after inhalation of the infectious aerosol; an initial host defense response of polymorphonuclear leukocytes and alveolar macrophages as a consequence of the pulmonary invasion; followed by the development of a specific humoral and cell-mediated immune response.<sup>1,9,12,13,15,16</sup>

### Technical or anatomic abnormalities

The presence of technical or anatomic abnormalities that lead to undrained fluid collections (blood, urine or bile, and/or lymph), devitalized tissues, and the need for the placement of drainage catheters and other foreign bodies that compromise mucocutaneous surfaces can contribute significantly to the risk of infection. Surgical misadventures, vascular access devices, and the need for prolonged respiratory support can all play a role here. Surgery in a transplant patient is the most unforgiving form of surgery that is encountered, with a surgical misadventure being highly associated with secondary invasive infection.<sup>1,2,5-7</sup>

### Darwinian factors

Darwinian factors are now being recognized as important contributors to the pathogenesis of certain infections. The most common example of this phenomenon is when broad-spectrum antibacterial therapy has been given in the setting of an anatomic problem that has not been corrected. *Candida* superinfection is a common result, requiring surgical correction as well as systemic antifungal therapy. Another version of Darwinian influences is when excessive amounts of growth

factors are present, thus favoring microbial overgrowth. Thus, excessive amounts of iron can result in zygomycosis, listeriosis, and other life-threatening infections, as well as an increase in the microbial burden in the case of a local staphylococcal wound infection.<sup>1,2,6,10,11</sup>

## Summary

Environmental exposures can occur within the community or in the hospital. In the community aerosols can be created and inhaled during the course of construction, gardening or farm work. Moulds such as *Aspergillus* can cause invasive disease following such exposures. In addition to such opportunistic infections, the endemic mycoses (*B. dermatitidis*, *C. immitis*, and *H. capsulatum*) can have a significant impact on the health of the transplant patient. These dimorphic fungi have a similar epidemiology and pathogenesis. They each are geographically restricted, growing in nature as a mould composed of a mesh of septate hyphae bearing conidia (the infectious particles) that are aerosolized during urban renewal projects, “clean-up” efforts, and explorations of sites where the soil is “enriched” with bird and animal droppings. Inhalation of aerosolized conidia will initiate clinical infection, with the tissue invasive form being yeast. The critical host defense is the cell-mediated response to tissue invasion, just that aspect of host defense most affected by the immunosuppressive drugs required for successful transplantation.<sup>9,12,16</sup>

Epidemiologic patterns of infection that occur are: primary infection, often with bloodstream dissemination; reactivation infection with the potential for secondary dissemination; and reinfection, again with the possibility of dissemination. Transplant patients are particularly vulnerable to reinfection, due to attenuation of previous immunity by the chronic immunosuppressive therapy.<sup>1,2,4</sup>

The net state of immunosuppression is a complex function that is made up of a number of factors. For those patients without viral infection, there is a high probability of an excessive environmental hazard that should be identified and corrected as quickly as possible, in order to prevent additional cases.<sup>1,2,4,6</sup>

## Clinical timetable for the occurrence of fungal infection in transplant patients

There is an important temporal component to the infections that occur post transplant; that is, different infections occur at a particular time after the transplant procedure and initiation of immunosuppression. Ultimately, the net state of immunosuppression (and the development of infection) is related to sustained therapy, the area under the curve, rather than the daily doses. The point to be emphasized is not that pneumonia can only occur at a specific time but rather that the restricted time period applies to the etiology.

The timetable can be useful in the following ways: in helping to generate a differential diagnosis in a patient who presents with a clinical illness that could be a manifestation of invasive infection; as a tool of infection control – exceptions to the timetable demand an explanation; and as the foundation of a variety of preventive strategies.

The three time periods that have been defined do not include the impact of prophylaxis. Both CMV and fungal

infection will be influenced by prophylactic antimicrobial therapy. A not uncommon result when this effort fails is to prolong the incubation period without affecting the incidence. As a general rule, the incubation period may be extended 2–6 months.<sup>1,2,4,6</sup>

## First month after transplant

There are three major causes of infection in this time period.

1. Infection in the recipient that was not eradicated prior to the transplant operation, and may be further exacerbated by surgery and post transplant immunosuppression. Although every patient should be carefully screened for active infection prior to the procedure, particular attention should be paid to those individuals already receiving immunosuppression prior to transplantation. Thus, we have seen patients with conditions such as Crohn's disease, primary biliary cirrhosis or a cardiomyopathy, in a desperate attempt to avoid transplantation, come to transplant receiving chronic corticosteroids, and with infection due to *Pneumocystis* or *Cryptococcus* already present. These infections can be a major problem in the early post transplant period.
2. Infection of donor origin, with bloodstream infection seeding the allograft prior to surgery. Parenchymal infection may contribute to infections such as histoplasmosis and the other endemic fungi in the recipient.
3. The most common infections in this time period are the same wound, pulmonary, vascular access or drainage catheter infections seen in normal hosts undergoing comparable surgery. The prime determinant is the technical skill with which the surgery and perioperative care are carried out.

Notable by their absence during this time period are infections with opportunistic fungal pathogens such as *Aspergillus*. Indeed, when such infection occurs, this should trigger a search for excessive environmental dangers.<sup>1,2,4,6</sup>

## One to six months after transplant

In this time period the net state of immunosuppression is particularly high, because of the sustained immunosuppression and the effects of the immunomodulating viruses. As a result, infections such as those due to *Aspergillus* species can occur in the absence of an unusually intense exposure. The most common cause of febrile syndromes during this period are the herpes group viruses, particularly CMV and human herpesvirus-6. Since these predispose to opportunistic infections such as those caused by fungi, *Listeria*, and *Nocardia*, a careful assessment for dual or sequential infection should always be a part of the clinical review once CMV is diagnosed.<sup>1,2,4,6</sup>

## More than six months after transplant

In this period the patients may be regarded as fitting into one of two general categories in terms of fungal infection. Approximately 80% of organ transplant patients will have had a good result from their transplant: good allograft function, maintenance immunosuppression, and freedom from viral infection. This group is at minimal risk for invasive fungal infection unless



an excessive environmental exposure has occurred. Mucocutaneous candidal infection and, uncommonly, asymptomatic pulmonary nodules due primarily to *Cryptococcus neoformans* are the major fungal concerns. The remaining patients have had a poorer outcome from transplantation: less satisfactory allograft function, too much acute and chronic immunosuppression, and, often, chronic or recurrent viral infection. This group is often characterized as “chronic ne’er do wells.” They are at the greatest risk of any transplant patient for disseminated cryptococcal infection, invasive aspergillosis, and, if the epidemiologic history is appropriate, systemic infection with one of the endemic mycoses.

## Fungal infections of particular importance for the organ transplant recipient

### The endemic mycoses

As previously discussed, transplant patients are particularly vulnerable to the endemic mycoses if an exposure has occurred. The salient epidemiologic and clinical characteristics of the different infections in this group of patients are very similar: the organisms are dimorphic, growing in the soil over a limited geographic area as a mould with the infectious particles, the conidia, liberated from the mould in an aerosol following a variety of manipulations of the infected soil. Inhalation of the conidia is the key step in initiating the infection, with a pulmonary portal of entry being characteristic of these organisms. Tissue invasion is accomplished by yeast forms. There is an initial polymorphonuclear leukocyte and alveolar macrophage response to these events, with a subsequent humoral and cell-mediated response. The key host defense is a specific cytotoxic T cell response. The combination of the initial non-specific inflammatory response with the specific response will limit the extent of the pulmonary infection, as well as access to the bloodstream and systemic infection. In transplant patients the attenuation of the specific immune response engendered by immunosuppressive therapy leads to an increased incidence and severity of progressive pulmonary and disseminated infection, as well as metastatic spread to sites such as the skin, the CNS, bones, and joints.<sup>8,9,12-17</sup>

#### Blastomycosis

*Blastomyces dermatitidis* grows as a mould on decaying wood. The geographic distribution of these organisms is similar to that of *H. capsulatum* (the midwest and southeastern United States). Human infection results following the inhalation of a conidia-laden aerosols. Clinically, pulmonary symptoms (cough, sputum production, chest pain, and dyspnea) predominate. Radiologic findings include non-specific infiltrates and hilar adenopathy. Disseminated infection not uncommonly results in metastatic skin involvement, with large nodular skin lesions that undergo necrosis and fibrosis. Genitourinary and skeletal infection are the other common sites of metastatic spread.

Blastomycosis is the least common of the systemic mycoses to infect the transplant recipient. Diagnosis usually requires biopsy for pathologic and cultural examination. Initial treatment usually requires an amphotericin preparation

administered intravenously, followed by oral itraconazole. The role of newer antifungals is still being defined.

#### Coccidioidomycosis

*Coccidioides immitis* is a dimorphic fungus which grows as a mesh of septate hyphae bearing the arthroconidia that initiate clinical disease following inhalation of an aerosol laden with these infectious arthroconidia. Within the mammalian host maturation of the arthroconidia into spherules, the definitive tissue pathogen, takes place.

The natural habitat of *C. immitis* is the desert soil of the Lower Sonoran life zone, an area whose climate features hot, dry summers and mild winters with moderate rainfall. Geographic areas that fit this description include the San Joaquin Valley of California, the southwestern United States, northern Mexico, and various sites in Central and South America. The arthroconidia can be so efficient at transmitting the infection that clinically important disease can occur many miles from an endemic area, due to wind or dust storm conditions, or exposure to dust on packages or clothing sent from the endemic sites. Major risk factors for serious clinical disease include the following: intensive immunosuppressive therapy, pregnancy, and non-Caucasian racial status. For example, on moving into an endemic area there is a 5% risk of developing primary infection during the first year of residence, with an additional annual infection rate of 2–3% per year. Dissemination, with a predominance of CNS infection, was noted in 75% of these individuals, with around two-thirds of these dying from this infection.<sup>6,8,16-20</sup>

A variety of clinical syndromes are associated with *C. immitis* infection. As previously discussed, primary infection in a non-immunosuppressed individual will probably cause hypersensitivity reactions; in contrast, in a transplant patient receiving immunosuppressive therapy, the hypersensitivity reactions are uncommon, and progressive pneumonia and/or bloodstream infection due to invasive disease are the rule. The most important manifestation of infection in transplant patients is meningitis. Typically, a diffuse granulomatous meningitis encases the base of the brain, resulting in hydrocephalus and cranial nerve palsies. Headaches and an impaired state of consciousness are common. Vasculitis can occur, resulting in focal neurologic findings, including aphasia, hemianopia, and hemiparesis, aneurysms and subarachnoid hemorrhage. CNS disease caused by this organism can be the first or only evidence of coccidioidomycosis, and vice versa. Other sites of metastatic infection include the skin, the skeleton, and the genitourinary tract.

The diagnosis of coccidioidomycosis can be difficult and should be regarded as being in a state of flux. Traditionally, KOH preparations of sputum, scrapings of skin or visceral tissues, or biopsies have yielded the diagnosis under the microscope. Cultures can be performed, but this approach is potentially dangerous if the diagnostic laboratory is inexperienced and/or appropriate protective biohazard equipment is not in place and utilized. Skin tests in immunosuppressed patients may be difficult to interpret. In contrast, rising titers of antibody, especially complement fixing antibody directed against *C. immitis*, are quite suggestive (although the possibility of cross-reacting antibodies can be seen with blastomycosis and histoplasmosis). Cerebrospinal fluid (CSF) in patients with meningitis usually reveals a lymphocytic pleocytosis, an elevated protein level and a low sugar (hypoglycorrhachia), and

increased intracranial pressure. Complement fixing antibodies in the CSF are of great diagnostic value. It is quite likely that in the next few years quantitative measurements of specific antigens and/or PCR detection of *C. immitis* nucleic acids will become the cornerstone of the diagnostic effort.

Antifungal chemotherapy for coccidioidomycosis is also in evolution. High-dose amphotericin therapy has been, and continues to be the standard of care. However, because cure is unlikely and relapse to be expected, we advocate a different approach: gain control with amphotericin and then switch to high-dose azole therapy. For this purpose, itraconazole has been used successfully. We prefer fluconazole for this long-term maintenance therapy, because of the greater reliability of absorption, pharmacokinetics, and lesser amounts of interaction with cyclosporine and tacrolimus. Ideally, preemptive fluconazole therapy should be prescribed for transplant patients with a past history of coccidioidomycosis or when the transplant care is being given in an endemic region. Once initiated, the fluconazole should be maintained for an indefinite period.<sup>8,16-20</sup>

### Histoplasmosis

*Histoplasma capsulatum*, the dimorphic fungus, grows well in soil enriched by the droppings of chickens, starlings, and bats. In the United States the center of activity is found in the Ohio and Mississippi river valleys, extending eastward into Virginia and Maryland. The inhalation of aerosolized organisms results in a patchy bronchopneumonia with a neutrophilic inflammatory response. This is followed by the development of specific cell-mediated immunity and, finally, by the development of epithelioid granuloma with Langhans' type giant cells, easily recognized on microscopic examination of the lung. Because of the impaired cell-mediated immune responses in transplant patients, systemic dissemination and/or progressive primary pneumonia are common, with infected mononuclear cells being efficient carriers of the infection to distant sites. Sites of extensive involvement are those organs with large numbers of reticuloendothelial cells: liver, spleen, lymph nodes, bone marrow, gut, and adrenal glands. In addition to these sites, mucocutaneous and CNS infection are not uncommon.<sup>5,6,8,9</sup>

Data presented by Wheat and his colleagues in Indianapolis, which is in the heart of the histoplasmosis belt, have shown that clinical disease occurs in 2–4% of renal transplant recipients, with a high percentage of these patients having disseminated disease. During an urban epidemic, this rate can exceed 10–15%. Although reactivation disease with secondary dissemination can occur, most such cases appear to be due to new exposures. Most clinical cases occur more than 6 months after transplant, particularly in those patients who have required greater than normal immunosuppression. Dissemination is the rule, with CNS infection, both parenchymal and meningeal, occurring in as many as one-quarter of cases. The clinical presentation of histoplasmosis is essentially identical to that of coccidioidomycosis: subacute onset of fever, respiratory complaints, metastatic infection, headache, and altered consciousness.<sup>8,9,12,14</sup>

## Opportunistic fungal infection

### Aspergillosis

Of the more than 200 species of *Aspergillus*, 95% of the clinical disease is caused by three species (*A. fumigatus*, *A. flavus*, and *A. niger*, with an occasional human case caused by

*A. nidulans*, *A. terreus*, *A. oryzae*, and *A. versicolor*). These organisms are ubiquitous in the environment and are particularly likely to cause infection in transplant patients in nosocomial or community settings in which construction activities result in the creation of an aerosol laden with *Aspergillus* conidia. There are several distinct clinical syndromes that can be produced following inhalation of the infectious aerosol.<sup>1,2,4</sup>

1. *Hypersensitivity syndromes*, including both asthma and extrinsic allergic alveolitis.
2. *Colonization syndromes*, in which the formation of fungal balls or mycetomas occurs in previously injured sinuses and, more commonly, in pulmonary cavities or sites of bronchiectasis. These fungal balls tend to cause irritation and inflammation. Life-threatening hemorrhage from the sites of the fungal balls can also occur.
3. *Allergic bronchopulmonary aspergillosis* is a clinical entity that combines aspects of both the hypersensitivity and colonization syndromes. Colonization of the tracheobronchial tree, an irritative cough, the appearance of "brown bits" in the sputum, and fleeting pulmonary infiltrates have been well described, including an 80% response to corticosteroids.
4. *Invasive aspergillosis* is the key response to this problem. Invasive aspergillosis is potentiated by steroids and/or neutropenia, and occurs only in patients who are significantly immunocompromised and/or exposed to a major environmental hazard. The consequences of invasive aspergillosis in this patient population are related to the vascular invasion that occurs: hemorrhage, infarction, and metastases. In a transplant patient, colonization with *Aspergillus* significantly increases the risk of subsequent invasion.
5. *Seminvasive aspergillosis* is an uncommon entity in which true immunocompromised is not present but subtle findings of hyperglycemia, liver disease, influenza, etc. are present and are thought to be associated with the slow progression of this necrotizing process. Vascular invasion is a minor part of this syndrome. Surgical ablation under cover with an azole drug appears to be the treatment of choice.

Other associations that merit comment include the presence of *Aspergillus* in marijuana, which can lead to invasive disease. Direct inoculation of damaged skin (e.g., vascular access sites, burns, and sites of inoculation) can likewise require treatment. In lung transplant recipients two additional forms of *Aspergillus* infection have been noted: infection of the suture line, with subsequent necrosis and disruption, and tracheobronchial disease.

Invasive aspergillosis, then, primarily occurs in situations of poor leukocyte function or sustained exposure to steroids. However, even patients whose net state of immunosuppression is minimal can develop invasive aspergillosis if the environmental exposure is great enough. An important corollary to this observation is that a single case of invasive aspergillosis occurring in the first month post transplant (a "golden period" during which opportunistic infection only occurs under conditions of unusually intense environmental exposure) should trigger a search for environmental hazards before more cases occur.<sup>1,2,4-6</sup>

Whatever the portal of entry, blood vessel invasion is the hallmark of invasive aspergillosis, resulting in the three

characteristics of this entity: tissue infarction, hemorrhage, and metastatic spread through the cardiovascular system. Of all the *Aspergillus* species, *A. fumigatus*, *A. flavus*, and *A. niger* account for virtually all cases, with *A. fumigatus* causing the majority of these.<sup>1,2,4-6</sup>

Clinically, invasive aspergillosis of the lungs, the most common presentation of invasive disease, can occur as the primary event or as a superinfection after pulmonary injury due to virus or bacteria, or pulmonary infarction. In as many as 50% of cases, metastatic spread has already occurred by the time of diagnosis, and a site of metastasis may be the first presentation of invasive disease. The typical radiologic finding of invasive aspergillosis of the lungs is focal lung disease, with either a nodule or a consolidation being present, often with cavitation. Unlike the patient with leukemia and aspergillosis, halo signs and air crescent signs are uncommon in organ transplant patients. Specific diagnosis requires a biopsy procedure. Other diagnostic approaches such as the galactomannan assay appear to be quite promising. If the early promise is confirmed, then early preemptive therapy will become an important part of patient management.<sup>1,2,4-6</sup>

The traditional view of *Aspergillus* syndromes is that there is little overlap; that is, a colonization syndrome never turns invasive or allergic bronchopulmonary disease does not turn invasive. It is now clear that on occasion there is “overlap” “or crossover.”

The availability of voriconazole now makes it possible to treat any evidence of invasive disease and, in many patients, avoid surgery. Combining voriconazole with surgery is also a useful strategy, preventing complications of surgical manipulation.<sup>1,2,4</sup>

The standard of care for invasive disease due to aspergillosis has long been a prolonged course of intravenous therapy. Sustained therapy of this sort was rendered difficult by the toxicities of this regimen: an acute infusion toxicity, with cytokine release, fever, rigors and malaise, chronic, progressive renal toxicity. Fortunately, there has been a major advance in the availability of alternative antifungal drugs.<sup>21-31</sup>

**Lipid-associated amphotericin B products** There are now three approved lipid-associated amphotericin products, whose use is approved by the Food and Drug Administration. The use of these drugs is clearly associated with a striking decrease in toxicity and a greatly increased cost. Issues such as optimal dose and duration and relative effectiveness are still being assessed. At present, a common strategy is to initiate therapy with conventional amphotericin and then switch to a lipid compound when the toxicity becomes evident, but is easily reversible. Amphotericin B, a polyene antifungal, acts by binding to fungal membrane sterols (most notably ergosterol) and causing a significant increase in fungal cell permeability, resulting in leakage of intracellular contents and cell death.

**Flucytosine** Flucytosine is mentioned here because it can only be used in conjunction with amphotericin. It has synergistic antifungal activity against yeast in the presence of an amphotericin preparation. Flucytosine can be administered orally and has a very useful pharmacokinetic profile (including the ability to penetrate the CNS, eye, and urinary tract). Single-step mutation to resistance is common and is the reason for using amphotericin to protect the flucytosine. Flucytosine has therapeutic effects related to its effects on nucleic

acid synthesis. Toxicities include the bone marrow and liver. Its primary therapeutic use is in the treatment of serious cryptococcal and candidal infection.

**Therapeutic azoles** These agents comprise the most important class of new therapeutic, antifungal drugs in the last two decades. Cytochrome P450-dependent 14- $\alpha$ -demethylase is the target of the azoles, causing a fungistatic effect on ergosterol synthesis.

The first of the new fungal agents to gain FDA approval, ketoconazole was the first of these drugs to reach the marketplace. It is available only in an oral preparation. The spectrum of activity is attractive; unfortunately, the pharmacokinetic profile is difficult (requires an acid pH in the stomach for absorption). Penetration of the CNS, eye, and urinary tract is unreliable. Hepatic toxicity is common, and it is primarily a drug of historic interest whose major use these days is as a third-line agent for prostate cancer.

Itraconazole requires gastric acidity for absorption and penetrates the CNS and urinary tract poorly. Formulation is an important aspect of the drug. Itraconazole in solution with  $\beta$ -hydroxydextrin is absorbed more reliably and completely than the parent drug. A dextrin formulation for parenteral use is now available. Itraconazole is reliably broad spectrum (including aspergillosis, “wrap-up” therapy for yeast and mould infection). Precise delineations of dose and duration are still being determined.

Fluconazole is the “best behaved” of the azoles, limited primarily by a spectrum of activity that includes *Candida* spp. and *Cryptococcus neoformans*. In addition, it has been shown to be useful as “wrap-up” therapy in the management of *C. immitis* infection, particularly that involving the CNS. The volume of distribution is quite good, including the CNS, eye, and urinary tract. Fluconazole has excellent bioavailability, with the same dose utilized orally and intravenously. Toxicity is less than with other antifungal drugs, consisting primarily of hepatic dysfunction and skin rashes. Fluconazole’s effect is primarily fungistatic. Therapy with an amphotericin preparation to gain control, followed by a prolonged course of oral fluconazole, is a commonly used strategy.

Voriconazole is a broad-spectrum azole, with useful activity against fluconazole-resistant *Candida*, *Aspergillus*, *Fusarium*, and *Pseudallescheria*. It is available both orally and parenterally, and should be considered the most effective drug currently available for aspergillosis. In addition to its utility against invasive aspergillosis, it can be useful against colonization syndromes as well, thus extending its range of clinical usefulness. There is also emerging evidence that combination therapy with other classes of anti-*Aspergillus* drugs may have value. Liver toxicity is more common than with fluconazole and needs to be carefully monitored. Particularly in patients being treated with high-dose intravenous therapy, a variety of transient visual complaints can occur, usually lasting a short period of time. These symptoms do not persist once the drug is discontinued and are thought to represent retinal dysfunction. In most respects this entity resembles the visual disturbances observed in some patients with digitalis toxicity.

Posaconazole resembles voriconazole in activity, efficacy, and toxicity, with one additional quality. It appears to be useful, particularly in combination with surgical resection, in the management of zygomycosis. Since there is some



evidence that zygomycosis is selected for by voriconazole, this property of posaconazole may be quite useful clinically. The full range of clinical uses for this drug is still being defined, as is the relative value of other azoles that are being studied.

**Echinocandins** Echinocandins are potent inhibitors of fungal cell wall synthesis, by means of their effects on  $\beta$ 1,3-glucan synthase. These agents bind rapidly to the fungal enzyme, causing rapid cell death of the fungus. The first of these, caspofungin, has been approved and appears to have useful activity against *Aspergillus*, *Candida*, some of the endemic fungi, and even *Pneumocystis*. Cryptococcal infection is not affected by the echinocandins. Whereas the other antifungal drugs exert their effects primarily through effects on ergosterol synthesis and function, thus affecting the fungal cell membrane, the echinocandins inhibit cell wall synthesis. Analogy has been made to the cell wall effects of penicillin in many bacteria. The hypothesis has been put forward that echinocandins are the “penicillins” of the antifungal world, and combination therapy with azoles and/or amphotericin formulations should increase penetration of the azoles and polyenes, and improve further antifungal efficacy. This hypothesis is currently being tested.<sup>21,22</sup>

### Candidiasis

Candidiasis is the most common form of fungal infection affecting the transplant recipient. The range of species and consequences of candidal replication are quite broad, ranging from mucocutaneous overgrowth to bloodstream infection and metastatic infection. There are three stages in the pathogenesis of candidal infection.

1. An increase in the *Candida* load in the gastrointestinal tract, mucocutaneous surfaces, and the female urogenital tract. The driving force is the level of glucose and glycogen at the site. This occurs in the face of broad-spectrum antibacterial therapy and in the setting of diabetes out of control, with the increased level of glucose functioning as an important growth factor.
2. A break in the mucocutaneous integrity at sites of glucose increase will promote bloodstream invasion.
3. Once penetration occurs, the key host defenses against candidal infection are adequate numbers of normally functioning polymorphonuclear leukocytes and intact cell-mediated immunity.

*Candida albicans* and *tropicalis* are the most likely species to cause clinical disease. However, in the face of person-to-person spread on the hands of medical personnel or exposures to fluconazole without correction of the anatomic abnormality associated with the occurrence of the infection, reinfection with *C. glabrata*, *C. krusei*, and other resistant strains causing disease is common.

Mucocutaneous infection, urinary tract infection, infection related to drains and catheters (including peritoneal dialysis catheters), and infection related to surgical procedures should be considered for anticandidal preemptive therapy, particularly when surgical manipulation is planned. Preemptive anti-candidal therapy should be considered when surgery is to be carried out in the presence of *Candida*. Similarly, vascular access devices, drainage catheters, and sites of a heavy microbial burden should also receive antifungal therapy. The

drugs to be considered are the those utilized for the other fungal species.

### Cryptococcosis

*Cryptococcus* is a ubiquitous organism that has a pulmonary portal of entry. Pulmonary disease, usually minimally symptomatic, is common and usually quite responsive to therapy. The lung findings vary from asymptomatic nodules to pneumonia. Postprimary dissemination via the bloodstream is common, with metastatic seeding of the meninges and/or the brain parenchyma (as well as sites such as the skin and skeletal system). CNS infection is the most important form of cryptococcal infection. Cryptococcal cellulitis may be the first evidence of disseminated infection. Most cases occur more than 6 months post transplant, particularly in patients who have been overimmunosuppressed, the chronic “ne’er do wells.” Therapy is given with an amphotericin preparation plus flucytosine, and then completed with fluconazole.

### Zygomycosis (syn. mucormycosis)

This is a rapidly progressive fungal infection that causes tissue infarction. This necrotizing infection is observed in the following situations.

1. Primary infection of the skin at a site traumatized by an extravasated, intravenous event or pressure dressings contaminated by *Rhizopus* sporangioconidia.
2. Primary pulmonary infections following inhalation of sporangiospores.
3. Rhinocerebral zygomycosis in which the fungal aerosol establishes disease in the nasal sinuses, with progressive extension into intracranial structures.

The clinical hallmark of zygomycosis is rapidly progressive, necrotizing infection, often with eschar formation in the skin and mucosa overlying involved tissues. Risk factors include acidosis, particularly diabetic ketoacidosis, steroids, overimmunosuppression, and devitalized tissues that can be secondarily infected. Therapy is with posaconazole +/- ablative surgery.

## Conclusion

The invasive fungal infections remain a major problem for transplant recipients, causing pneumonia and bloodstream infection at a considerable cost and with greater consequences if early diagnosis and aggressive therapy have not been accomplished. There has been a major improvement in the treatment of fungal infection, with there being three separate classes of drugs (lipid-associated amphotericin drugs, antifungal azoles, and echinocandins) that can be utilized. Particularly appealing is the possibility that combination therapy, bringing together different mechanisms of action, will result in even better and more effective therapy. Early diagnosis, as with the galactomannan assay or a PCR-based technique, will improve the therapeutic results. Better diagnostics will also help. High-definition chest CT is proving to have a role in early diagnosis which is an example of utilizing existing technology in an innovative fashion. One word of caution: false-positive galactomannan assays are seen with piperacillin/tazobactam and amoxicillin/clavulanate up to a week after discontinuing the antibiotics.

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# Fungal infections in pediatric patients

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Fungal infections occur either in healthy immunocompetent hosts or as opportunistic infections in patients at risk. In the first category the endemic mycoses and the dermatophytoses are the best examples, whereas in the second category the invasive fungal infections predominate.

Invasive fungal infections have evolved into important causes of morbidity and mortality in children with severe underlying illnesses. Irrespective of age and underlying condition, these infections remain difficult to diagnose and responses to treatment depend on early diagnosis and restoration of host defenses. For more than three decades, options for antifungal chemotherapy have been limited to amphotericin B with or without flucytosine. Recent years, however, have witnessed an expanded clinical experience of antifungal triazoles and development of less toxic lipid amphotericin B formulations and echinocandins. New diagnostic modalities have been investigated, aiming at earlier diagnosis. Children, in particular neonates and young infants, represent a unique patient population, with regard to both patterns of invasive fungal infections and the disposition of antifungal agents. This chapter therefore discusses the unique features of fungal infections and problems of diagnostic modalities in children as well as antifungal therapeutics in this population.

## Invasive fungal infections in pediatric patients

Invasive fungal infections are caused by a large variety of fungal pathogens and are found in patients of all ages (Table 22-1). At first look, the patterns of these infections in neonates, infants, children and adolescents may appear similar to those encountered in adults. However, the pediatric groups display important differences in host biology and defense, and are characterized by an array of predisposing conditions as well as certain unique features that may require different approaches to management.

### Host biology

Biologic characteristics that may be unique to pediatric age groups include specific anatomic, physiologic, and immunologic aspects.

Anatomic considerations are important throughout infancy, but particularly in preterm neonates. Due to the decreased keratin layer and reduced thickness of the skin, the application of medical devices and the moist environment, preterm neonates have a particular susceptibility to developing primary cutaneous aspergillosis and zygomycosis.<sup>1-3</sup> Similarly, the extremely tenuous wall structures of the gastrointestinal tract lead to a unique propensity to primary invasive gastrointestinal infections by the same agents with precipitous gastrointestinal perforation, a pattern that is relatively uncommon in other settings.<sup>1,2</sup> The comparably small diameter of blood vessels provides a nidus for catheter-associated *Candida* thrombophlebitis, thrombosis and endocarditis;<sup>4-6</sup> life-threatening *Candida* laryngitis and epiglottitis may occur in immunocompromised infants and young children for the same anatomic reasons.<sup>7</sup>

In neonates, physiologic differences such as the larger fractional water content, the smaller plasma protein fraction, relatively larger organ volumes, and the functional immaturity of hepatic metabolism and renal excretion may all lead to profound differences in drug distribution, metabolism, and elimination.<sup>8</sup> Moreover, the yet incomplete blood-brain barrier, in addition to having pharmacologic consequences on drug penetration, may also be one reason for the relatively enhanced risk of the newborn to develop clinically overt meningoencephalitis, an otherwise unusual complication of invasive *Candida* infections.<sup>9,10</sup> Infants and younger children continue to exhibit differences in the relative proportion of body water, adipose tissue, and organ volumes; as compared to many adults with subclinical age-related organ impairment; however, these populations may have larger functional reserve capacities of liver and kidney.<sup>8</sup>

Specific immunologic characteristics in neonates include a functional immaturity of mononuclear and polymorphonuclear phagocytes and T lymphocytes<sup>11</sup> as well as a possibly increased susceptibility to the immunosuppressive effects of corticosteroids.<sup>1</sup> These deficiencies may render neonates susceptible to nosocomially acquired opportunistic fungal infections. The still developing cellular immunity may also explain the occurrence of overwhelming infections by *Histoplasma capsulatum*<sup>12</sup> and possibly other endemic fungi in infants. The pediatrician may also encounter neonates and infants who present with superficial or invasive fungal infections as one of the first manifestations of



**Table 22-1** Fungal pathogens that cause invasive diseases

Opportunistic yeasts	Dematiaceous moulds
• <b><i>Candida albicans</i></b>	• <i>Pseudallescheria boydii</i>
• <b>Non-<i>albicans</i> <i>Candida</i> spp., in particular <i>C. parapsilosis</i></b>	• <i>Dactylaria</i> spp.
• <i>Cryptococcus neoformans</i>	• <i>Alternaria</i> spp.
• <i>Trichosporon beigeli</i>	• <i>Curvularia</i> spp.
• <i>Malassezia</i> spp. and others	• <i>Bipolaris</i> spp.
	• <i>Wangiella</i> spp. and others
Opportunistic moulds	Endemic dimorphic moulds
• <b><i>Aspergillus</i> spp.</b>	• <i>Histoplasma capsulatum</i>
• <i>Fusarium</i> spp.	• <i>Coccidioides immitis</i>
• <i>Zygomycetes</i> spp.	• <i>Blastomyces dermatitidis</i>
• <i>Acremonium</i> spp.	• <i>Sporothrix schenckii</i>
• <i>Paecilomyces</i> spp.	• <i>Paracoccidioides brasiliensis</i>
• <i>Trichoderma</i> spp. and others	• <i>Penicillium marneffei</i>

Most prevalent organisms marked in bold.

a congenital T cellular immunodeficiency or of chronic granulomatous disease (CGD).<sup>13,14</sup> In older children and adolescents, genetic illnesses such as cystic fibrosis or B cell disorders, that lead to chronic recurrent airway infections and lung destruction, may result in allergic airway disease, aspergilloma, and sometimes invasive mould infections.<sup>15</sup>

## Populations at risk

The pediatric populations at risk can be defined by specific predisposing defects in host defenses and several additional, non-immunologic factors. In general, deficiencies in the number or function of phagocytic cells are associated with invasive infections by opportunistic fungi, such as *Candida albicans* and non-*albicans* *Candida* spp., *Trichosporon* spp., *Aspergillus* spp., *Fusarium* spp., *Zygomycetes* and a large variety of other, less frequently encountered yeasts and moulds. In contrast to that, deficiencies or imbalances of T lymphocyte function are followed by mucocutaneous candidiasis and invasive infections by *Cryptococcus neoformans* and dimorphic moulds. Non-immunologic factors include exposure to the organism, pre-existing tissue damage, and, limited to *Candida* spp., presence of indwelling vascular catheters, colonization of mucous membranes, use of broad-spectrum antibiotics and/or parenteral nutrition, and complicated intraabdominal surgery.<sup>16</sup>

In extension of this classification, the pediatric populations at risk for invasive fungal infections include neonates, in particular preterm neonates, pediatric patients with congenital immunodeficiencies involving phagocytosis and T lymphocyte function (most frequently CGD and chronic mucocutaneous candidiasis (CMC)), pediatric patients with acquired immunodeficiencies such as human immunodeficiency virus (HIV) infection, cancer, bone marrow or solid organ transplantation, and immunosuppressive therapy with corticosteroids, as well as children of all age groups beyond the neonatal period who are hospitalized for severe acute illnesses or who have chronic destructive lung diseases or are otherwise healthy (Table 22-2).

**Table 22-2** Pediatric populations at risk for invasive fungal infections

- Neonates
- Infants
- Children with Congenital Immunodeficiencies
  - Defects of phagocytic host defenses
  - Defects of specific cellular host defenses
- Children with Acquired Immunodeficiencies
  - Iatrogenic immunosuppression
  - Treatment for cancer
  - HIV infection
- Children with Acute Illnesses
- Children with Chronic Airway Diseases
- Otherwise Healthy Children

## Epidemiology and presentation

The neonate

*Candida* spp. colonize the vaginal tract of approximately 30% of pregnant women; very rarely, they can become the cause of chorioamnionitis and intrauterine infection manifested as congenital candidiasis.<sup>17</sup> In healthy neonates, *Candida* rapidly colonizes the mucocutaneous surfaces and it may cause thrush and diaper dermatitis.<sup>18</sup> In hospitalized, ill neonates, *Candida* has evolved as an important cause of life-threatening invasive infections, particularly in very low birth weight (VLBW) and extremely low birth weight (ELBW) infants. Risk factors for nosocomial colonization of *Candida* spp., particularly *C. parapsilosis*, are low birth weight, long stay in the neonatal intensive care unit (NICU), H<sub>2</sub> blockers, third-generation cephalosporins and delayed enteral feedings, which alter gastrointestinal tract ecology, facilitating colonization.<sup>19,20</sup> *Candida* spp. now account for 9–13% of all bloodstream isolates in NICU.<sup>21–24</sup> Recent case series indicate that invasive candidiasis occurs in up to 5% of infants with a birth weight of <1500 g (VLBW) and in 8–28% of infants with a birth weight of <1000 g (ELBW); the crude mortality associated with these infections ranges from 15% to 30% with an attributable mortality of 6–22% despite appropriate therapy.<sup>25,26</sup> Thus, while *Candida* is the third most common pathogen causing bloodstream infection in the NICU, it is the second most frequent cause of death due to sepsis. Invasive candidiasis in preterm infants is most commonly due to *C. albicans* and *C. parapsilosis*<sup>23,27</sup> and associated with vascular catheters, use of broad-spectrum antibiotics and corticosteroids, prior mucocutaneous colonization, and parenteral hyperalimentation.<sup>28,29</sup>

Most neonates with systemic candidiasis are symptomatic at the onset of their disease and present with signs and symptoms that are virtually identical to those of non-fungal causes. Among deeply invasive infections, cutaneous, renal, pulmonary, and cerebral involvement are disproportionately common,<sup>10,16</sup> and *Candida* is increasingly recognized as a causative agent of infections associated with ventricular shunts and drains.<sup>30</sup> Numerous outbreaks have been reported, which underscore the importance of appropriate infection control measures for prevention of these infections.

*Malassezia* spp., lipophilic commensal yeasts that colonize the human skin and are the agents of pityriasis, can gain

access to the bloodstream via percutaneous vascular catheters to cause a potentially fatal systemic infection in premature infants receiving parenteral nutritional lipid supplements. Similar to *Candida*, the most probable mode of acquisition is via the hands of healthcare workers<sup>31</sup> but direct contamination through contaminated intravenous solutions and catheters has also been reported.<sup>32</sup> Special media containing olive oil are required for isolation.

*Trichosporon* spp., formerly known as one species, *Trichosporon beigelii*, now consisting of several species, have been reported to cause invasive infection in neonates. The most frequent isolate is *Trichosporon asahii*.<sup>33</sup> Among 14 published cases reviewed, the spectrum of disease ranged from colonization of a venous catheter to disseminated infection in 10 (71%). Resistance to amphotericin B has been reported<sup>34</sup> and may be a contributing factor to the poor outcome in patients with disseminated disease. Although azoles may be alternative efficacious agents, published data indicating efficacy in neonates are limited.

Infections by *Aspergillus* spp. and Zygomycetes are rare. In the neonatal setting, they tend to have a predilection for the skin and, in the case of Zygomycetes, for the gastrointestinal tract, resulting in necrotizing skin lesions and devastating necrotizing enterocolitis, respectively. Potential sources of the organisms are contaminated water, ventilation systems, and dressing materials or infusion boards.<sup>1,35</sup> Forty-four cases of invasive aspergillosis had been reported in children of  $\leq 3$  months of age; most of these infants had invasive pulmonary (23%), primary cutaneous (25%) or disseminated aspergillosis (32%).<sup>1</sup> Prematurity, CGD, and a complex of diarrhea, dehydration, malnutrition, and invasive bacterial infections accounted for the majority of underlying conditions (82%). Only a few patients were neutropenic, but at least 41% had received corticosteroids. While all other forms of the disease mainly occurred in term infants, cutaneous and gastrointestinal aspergillosis occurred almost exclusively in preterm neonates. Disseminated disease was uniformly fatal, whereas among 17 cases of neonates with primary cutaneous aspergillosis recently reviewed, nine (53%) died.<sup>3</sup> However, patients who had disseminated or cutaneous disease and received appropriate therapy had over 70% survival.

Similarly, 33 cases of invasive zygomycosis have been reported in neonates.<sup>2</sup> The predominant underlying condition was prematurity (70%) and 70% of the cases were due to *Rhizopus* spp. The most common patterns of zygomycosis were disseminated (49%), gastrointestinal (24%), deeply extended (15%) and cutaneous (6%). Except for cutaneous, the other forms were highly lethal with a mortality >85%.

A few cases of other mould infections have also been reported in the literature. Invasive mould infections in the setting of neonatal medicine should be considered in infants with expanding, necrotizing skin lesions or gastrointestinal perforation. Surgical debridement is essential in most cases.<sup>1</sup>

### The infant

Disseminated histoplasmosis is a classic example of the potentially dismal course of a primary infection by an endemic fungus in apparently healthy infants who were exposed to the organism. The disease is fatal if not detected and treated. Its clinical manifestations include prolonged fevers, failure to thrive, hepatosplenomegaly, pancytopenia and, ultimately,

disseminated intravascular coagulation and multiorgan failure.<sup>12,36</sup> Not much is known about blastomycosis and coccidioidomycosis in this age group, but ultimately fatal cases have been reported.<sup>37,38</sup>

### Children with congenital immunodeficiencies

Among the phagocyte defect syndromes, myeloperoxidase (MPO) deficiency is the most common entity. While MPO-deficient cells have only minor microbicidal abnormalities against bacteria in vitro, killing of *Candida* spp. is highly deficient and may serve as an explanation for invasive *Candida* infections reported in some patients with this disorder.<sup>39</sup> Chronic granulomatous disease of childhood is a genetically diverse congenital disorder of the NADPH oxidase complex that is associated with an inability of phagocytic cells to provide antimicrobial oxidants and to kill ingested microorganisms or extracellular fungal elements. It is the prime example of an inherited immune disorder with a high risk of invasive fungal infections; at the same time, it serves as a paradigm for the importance of phagocytic killing in the defense of infections by opportunistic moulds.

Invasive fungal infections, particularly invasive aspergillosis, may repeatedly complicate the course of this disorder, accounting for an estimated lifetime incidence of between 16% and 40%.<sup>13,14,40</sup> The most frequent sites of infection are the lungs; however, osteomyelitis is a particularly common form of invasive infection due to *Aspergillus* spp. and other filamentous fungi.<sup>40</sup> *Aspergillus fumigatus* and *Aspergillus nidulans* are among the most common fungi causing infection in these patients. *Scedosporium* spp. and *Paecilomyces* spp. are more rare fungal species isolated from these patients.<sup>14,41</sup> Galactomannan detection assay, a helpful early diagnostic tool in adults with hematologic malignancies, has low sensitivity in these children.<sup>42</sup>

Whether interferon- $\gamma$  or prophylactic antifungal triazoles such as itraconazole may reduce the frequency of fungal infections is not yet well established; however, there is indirect evidence that continuous administration of interferon- $\gamma$  has in vivo antifungal activity. In addition, daily administration of itraconazole to 39 patients with CGD was safe and prevented fungal infections, as compared to placebo. Treatment of mould infections, if they occur, is protracted and consists of antifungal chemotherapy, interferon-gamma, and appropriate surgical interventions.<sup>43-45</sup>

The role of immunoglobulins and antibodies in antifungal host defenses is important against cryptococcosis and possibly mucosal and invasive candidiasis,<sup>46</sup> but it is not well understood for other fungal infections. Children with inherited deficits of B lymphocytes appear not at increased risk for fungal infection, unless there is a concomitant disorder of T lymphocytes or phagocytes. This includes individuals with the X-linked hyper-IgM syndrome and patients with the hyper-IgE (Job) syndrome, which is associated with mucocutaneous candidiasis and, possibly, with cryptococcosis and invasive aspergillosis.<sup>14</sup>

Inherited immunodeficiencies involving number or function of T lymphocytes predispose to mucocutaneous and occasionally invasive candidiasis, and conceptually to cryptococcosis and histoplasmosis. Severe combined immunodeficiency (SCID) and severe types of thymic hypoplasia (DiGeorge syndrome) are medical emergencies of the neonatal period that can be managed

successfully only with hematopoietic stem cell transplantation or thymus transplantation, respectively.<sup>47,48</sup> Refractory mucocutaneous candidiasis is a hallmark of these disorders and can therefore be an important clue to the appropriate immunologic work-up. Chronic mucocutaneous candidiasis is a less severe congenital immunodeficiency with an impaired T cell response to *Candida* antigens.<sup>49</sup> It is characterized by chronic recurrent candidiasis of nails, skin, perineum, and oropharynx and may be idiopathic or associated with either the polyendocrinopathy syndrome (PEPS) type I or the hyper-IgE syndrome.<sup>50-52</sup>

#### Children with acquired immunodeficiencies

**Iatrogenic immunosuppression** Treatment with pharmacologic dosages of corticosteroids rapidly provides a functional impairment of phagocytosis by mononuclear and polymorphonuclear leukocytes. Similar to adults, such therapy is one of the most important reasons for the increased susceptibility to invasive fungal infections of children with immunosuppressive therapy for immunologic disorders, following solid organ transplantation, and following engraftment after bone marrow transplantation.<sup>16,53,54</sup>

**Cancer.** While current treatment for pediatric cancers is curative in most instances, highly dose-intensive chemotherapy regimens and aggressive supportive care measures also result in profound impairments of host defenses. Prolonged, profound neutropenia is the single most important risk factor for opportunistic fungal infections in children and adolescents with cancer. Other well-known, but notable risk factors include chemotherapy-induced mucositis, extended courses of broad-spectrum antibiotics, the presence of indwelling central venous lines and, particularly in children with acute leukemia, the therapeutic use of corticosteroids.<sup>55</sup>

Oropharyngeal candidiasis may occur in up to 15% of children undergoing intensive chemotherapy or bone marrow transplantation despite various forms of topical or systemic antifungal prophylaxis.<sup>56</sup> Esophageal candidiasis is also common, even in the absence of conspicuous oropharyngeal candidiasis,<sup>16</sup> and *Candida* epiglottitis and laryngitis may emerge in neutropenic children as life-threatening causes of airway obstruction.<sup>7</sup>

Similar to the adult cancer population, *Candida* spp. and *Aspergillus* spp. are the most common causes of invasive fungal infections in children with cancer. Invasive candidiasis in neutropenic children may present as candidemia, acute disseminated candidiasis, and deep single organ candidiasis. Its overall frequency in children with high-risk leukemias and/or bone marrow transplantation is between 8% and 10%; the crude mortality associated with these infections is at least 20% and close to 100% in patients with persistent neutropenia.<sup>57-59</sup> Catheter-associated fungemia is most commonly caused by *C. albicans*, but non-*albicans* *Candida* spp., particularly *C. parapsilosis*, and previously uncommon yeast pathogens are increasingly encountered.<sup>60</sup> Whether the primary source of fungemia or a target for attachment of circulating organisms, the intravascular catheter with the *Candida* biofilm formed on its plastic surface<sup>61</sup> serves as a source for continued seeding of the bloodstream and should be removed whenever feasible.<sup>62,63</sup>

Acute disseminated candidiasis occurs typically in neutropenic children and manifests with persistent fungemia, hemodynamic instability, multiple cutaneous and visceral lesions and high mortality despite antifungal therapy.<sup>16,59</sup>

*Candida albicans* is the most frequent cause, although *Candida tropicalis* has been increasingly implicated as an important pathogen in neutropenic children. Nineteen children were reported who were treated for leukemia in which *C. tropicalis* infections developed.<sup>64</sup> Fungemia without meningitis in 11 children was treated successfully, whereas meningitis in seven children was uniformly fatal, underscoring that meningitis is a critical factor for outcome of this infection. Chronic disseminated candidiasis typically presents with fever despite neutrophil recovery, often coupled with right upper quadrant abdominal pain, and increased alkaline phosphatase levels.<sup>65</sup> Imaging studies demonstrate multiple lesions in liver, spleen, and other organs that correspond morphologically to large granulomas with extensive chronic inflammatory reaction. Treatment is protracted<sup>16</sup> but may not necessarily require the interruption of anticancer therapy, provided that the disseminated infection has stabilized or is resolving.

Invasive aspergillosis (IA) has emerged as an important cause for morbidity and mortality in children with hematologic malignancies or undergoing bone marrow transplantation; more recent pediatric series indicate a frequency of 4.5–10% in this setting with an associated crude mortality of 40–94%.<sup>66-68</sup> A recent study found that aspergillosis occurred with an annual incidence of 437/100,000 (0.4%) among hospitalized immunocompromised children in the USA during 2000.<sup>69</sup> The overall in-hospital annual mortality of immunocompromised children with IA was 18%. The disease is almost absent in children treated for solid tumors, underscoring the role of prolonged neutropenia and corticosteroid therapy in its pathogenesis.<sup>68</sup> As in adults, *A. fumigatus* is the most frequent cause with *A. flavus* and *A. terreus* following as more rare causes of IA in children.

Similar to the adult setting, the lungs are the most frequently affected sites, and disseminated disease is found in approximately 30% of cases.<sup>66</sup> While paranasal sinus aspergillosis appears to be less common than in adults,<sup>70,71</sup> primary cutaneous aspergillosis has been preferentially reported in the pediatric setting in association with lacerations by arm boards, tapes, and electrodes and at the insertion site of peripheral or central venous catheters.<sup>71,72</sup> With combined surgical and medical therapy, primary cutaneous aspergillosis has a comparatively more favorable prognosis.<sup>71</sup>

CNS aspergillosis in infants and children predominantly presents as single or multiple brain abscesses and has significantly better outcome compared to published adult data, similar to invasive aspergillosis overall. A systematic review of 90 cases of CNS aspergillosis in infants and children published up to 2005 found that leukemia was the most frequent underlying disease and the outcome has significantly improved recently, with published mortality as low as 40%.<sup>73</sup>

Indeed, the outcome of invasive aspergillosis in children with hematologic malignancies may not be as dismal as in adults. Apart from recurrent or refractory cancer, the main obstacles to a successful outcome were failure to diagnose the invasive aspergillosis during life and, in patients with established diagnosis, catastrophic pulmonary or cerebral hemorrhage.

Similar to histoplasmosis,<sup>74</sup> cryptococcal meningoencephalitis and pneumonitis are rare opportunistic infections in children with cancer. In patients with pediatric sarcomas, however, pulmonary cryptococcosis may be a differential diagnosis of lung metastasis.<sup>75</sup>



During recent years, previously uncommon fungal pathogens have been increasingly recognized to cause systemic infection in neutropenic patients.<sup>76</sup> Particularly notable among the yeast-like organisms is *Trichosporon asahii*, a normal human commensal and the agent of white piedra. Trichosporonosis in neutropenic patients presents in a similar way to systemic candidiasis, with fungemia and disseminated infection, and carries a high mortality.<sup>60</sup> *Trichosporon asahii* is often resistant to the fungicidal effects of amphotericin B but may be amenable to antifungal azoles, especially voriconazole.<sup>34,77,78</sup>

*Fusarium* has emerged in some institutions as the second most common filamentous pathogen after *Aspergillus*, with *Fusarium solani* being the most frequent isolate.<sup>76,79</sup> Like the latter, the airborne organism is highly angioinvasive and leads to hemorrhagic infarction. Nosocomial outbreaks of infection due to this organism have been described.<sup>80</sup> *Fusarium* is among the few filamentous fungi that cause detectable fungemia and metastatic skin lesions are a hallmark of disseminated fusariosis. A clinical stabilization can sometimes be achieved with high dosages of amphotericin B, but rapid recovery from neutropenia is always a prerequisite for survival.<sup>60,79</sup>

Among the filamentous fungi, the Zygomycetes are notorious for their propensity to invade blood vessels, a rapidly deteriorating clinical course, and clinical refractoriness to antifungal therapy; the most common clinical presentations in the neutropenic host are rhinocerebral, pulmonary, cutaneous, and disseminated infection. A systematic review of 157 cases found neutropenia (18%), prematurity (17%), diabetes mellitus (15%), ketoacidosis (10%) and no apparent underlying condition (14%) as most frequent underlying conditions.<sup>81</sup> The most common patterns of zygomycosis were cutaneous (27%), gastrointestinal (21%), rhinocerebral (18%) and pulmonary (16%). *Rhizopus* spp. and *Mucor* spp. were most commonly identified. Mortality in patients without antifungal therapy was higher than in those with therapy (88 vs 36%). While cerebral, gastrointestinal and disseminated zygomycosis were associated with mortality rates of 88–100%, cutaneous zygomycosis was followed by no mortality at all. In a multivariate regression analysis, independent risk factors for death were disseminated infection and age <1 year. Antifungal therapy and particularly surgery significantly reduced risk of death by 92% and 84%, respectively.

**HIV infection** Children are recognized as one of the most rapidly expanding populations worldwide infected with HIV; mucosal as well as invasive fungal infections are major causes of morbidity and mortality in advanced stages of the disease.<sup>82</sup>

Oropharyngeal candidiasis (OPC) has been the most prevalent opportunistic infection in HIV-infected children and can occur in virtually all patients at some time during the course of their disease. Esophageal candidiasis has occurred in about 8% of these children and is associated with recurrent OPC, low CD4+ counts, and use of broad-spectrum antibiotics.<sup>83</sup> Unless significant immunologic reconstitution can be achieved, oropharyngeal and esophageal candidiasis has an exceedingly high propensity to recur. With the advent of HAART, OPC is not as prevalent as it was previously. The chronic use of fluconazole under these circumstances has been associated with the emergence of fluconazole-resistant *Candida* strains; it has been shown that such resistant strains can be exchanged among HIV-infected family members.<sup>84</sup> Children with HIV infection may develop candidemia or disseminated

candidiasis as a nosocomial infection during prolonged hospitalization for complicated medical problems. However, with the increasing use of outpatient treatments, the initial presentation of deeply invasive candidiasis may be shifting to the outpatient setting.

In a study in HIV-infected children, candidemia presented as community-acquired infection that was associated with ambulatory total parenteral nutrition and intravenous therapy via indwelling central venous lines.<sup>85</sup> Univariate and multiple logistic regression revealed that the prolonged presence of a central venous catheter was the most important risk factor for fungemia. In this study, non-*albicans* *Candida* spp. accounted for almost 50% of all isolates. A high rate of survival (95%) from fungemia without posttherapeutic sequelae was obtained by early detection, administration of amphotericin B, and removal of the vascular catheter.<sup>85</sup>

HIV-related impairment of phagocytosis by leukocytes<sup>86</sup> greatly contributes to the increased susceptibility of patients with advanced HIV infection to invasive aspergillosis.<sup>87</sup> Invasive aspergillosis was diagnosed in seven (1.5%) of 473 HIV-infected children followed at the Pediatric Branch of the National Cancer Institute from 1987 to 1997. Invasive pulmonary aspergillosis occurred in five, and aspergillosis of the skin and adjacent soft tissues in two patients. All patients had low CD4+ counts (median, 2/ $\mu$ l; range, 0–338). Neutropenia (<500/ $\mu$ l) lasting for >7 days or corticosteroid therapy were encountered in only two patients. Consistent with the experience in other immunocompromised children,<sup>71</sup> patients with cutaneous aspergillosis were diagnosed during life and successfully treated, whereas diagnosis of pulmonary aspergillosis was made antemortem in only one patient.<sup>87</sup>

Compared to the adult population, HIV-infected children have lower rates of cryptococcal infections, and, with the exemption of disseminated penicilliosis,<sup>88</sup> data on histoplasmosis and other endemic fungal infections are very limited.<sup>82</sup> With an estimated 10-year point prevalence of 1%,<sup>89</sup> cryptococcosis appears to be an infrequent opportunistic infection in HIV-infected children. It is associated with low CD4+ counts and, in the majority of cases, a previous AIDS-defining illness and older age; the clinical presentation may be subtle to fulminant, and may include unexplained fever and mostly diffuse central nervous and/or respiratory symptoms.<sup>90</sup> A review of 30 of an approximate total of 50 published cases indicated a crude mortality of 23% within the first month after diagnosis.<sup>89</sup>

#### Children with severe acute illnesses

Incidence of candidemia in pediatric patients follows the same pattern of increase seen in adults, but the rate of increase is higher. Pediatric patients in critical condition, particularly young infants, are especially vulnerable to invasive *Candida* infections, partly because of their age and severe underlying disease and partly because of the invasive procedures used. Invasive procedures, indwelling vascular and urinary catheters, use of broad-spectrum antibiotics and corticosteroids, mechanical ventilation and parenteral feeding as well as length of stay and severity of the underlying condition all contribute to a heightened risk of deeply invasive *Candida* infections in critically ill patients requiring intensive care. While few data are available for general pediatric intensive care units, studies in adults have confirmed the high frequency of nosocomial *Candida* infections in this setting.<sup>91,92</sup>

*Candida* spp. are currently the fourth most common cause of bloodstream infections in ICUs<sup>91</sup> and account for up to 17% of microbiologically documented infections.<sup>92</sup> Mirroring the general epidemiologic trend, more than half of such infections are now due to non-*albicans* *Candida* spp., particularly *C. parapsilosis* and *C. tropicalis*.<sup>91</sup> Some of the non-*albicans* *Candida* spp., such as *Candida krusei* and *Candida glabrata*, are intrinsically or potentially resistant to antifungal agents. The increased use of antifungal agents in immunocompromised patients, mainly prophylactically, is considered to be the strongest contributory factor to the changes in species distribution, which have subsequently affected the mortality and choice of empiric treatment. Prompt removal of lines and initiation of antifungal treatment are the milestones of management. Conventional amphotericin B remains a useful drug, whereas its lipid formulations and triazoles are effective agents. Novel antifungal agents, such as second-generation triazoles and echinocandins, exhibit potential as alternative agents in critically ill children with invasive candidiasis. Although response rates are still far from satisfactory, improved understanding of risk factors and new treatment options promise a better future outcome.

Zygomycosis may develop in the settings of neutropenia, corticosteroid therapy, bone marrow or solid organ transplantation, burn, and deferoxamine therapy for iron or aluminum overload states.<sup>81</sup> Zygomycosis in children occurs in other distinct settings as well: juvenile-onset (type I) diabetes mellitus, particularly with uncontrolled diabetic ketoacidosis, and congenital aminoaciduria.<sup>16</sup> For example, among 41 reported cases of rhinocerebral zygomycosis in children beyond the neonatal age, 20 (49%) had diabetes mellitus. Rhinocerebral zygomycosis usually begins as an infection of the paranasal sinuses, which progresses to invade the orbit, retroorbital region, cavernous sinus and brain. Infants and children with iron overload diseases and deferoxamine treatment are at risk for invasive zygomycosis.

#### Children with chronic airway diseases

Fungal infections may also occur in children and adolescents with chronic sinopulmonary infection and lung destruction, as it may be associated with congenital B cell defects, the hyper-IgE syndrome, and, most commonly, cystic fibrosis. Non-invasive fungal diseases associated with the colonization of the respiratory tract by *Aspergillus* spp. and other moulds such as allergic bronchopulmonary aspergillosis and aspergilloma formation clearly predominate in this setting. However, invasive pulmonary mould infections have been reported.<sup>51,93,94</sup>

#### Children with no underlying disease

Invasive fungal infections due to saprophytic fungi are very rare in otherwise healthy hosts. One example is the infection due to *Scedosporium apiospermum* and its teleomorph *Pseudallescheria boydii* which under specific conditions, such as near-drowning, may cause therapy-refractory and life-threatening infections. Nine pediatric cases published were recently reviewed, all in immunocompetent children, with a median age of 3 years (range 1.3–15 years). Pathologic findings included multiple brain abscesses (67%), meningoencephalitis (44%), cerebral vasculitis (22%), endophthalmitis (22%) and osteomyelitis (11%). *Scedosporium* spp. are highly resistant to amphotericin B and antifungal treatment is very difficult. It is usually combined with neurosurgery. Mortality was 67%.<sup>95</sup>

## Dermatophytoses and other superficial fungal infections

Dermatophytosis is caused by *Microsporon* spp., *Trichophyton* spp., and *Epidermophyton floccosum*. While tinea capitis, tinea corporis, and tinea facialis are not infrequently encountered in children, onychomycosis is unusual. The most common agent of tinea capitis in North America is the anthropophilic fungus *Trichophyton tonsurans*<sup>96</sup> whereas in several parts of Europe the zoophilic fungus *Microsporum canis* predominates.<sup>97</sup> When due to anthropophilic dermatophytes, tinea capitis is readily spread among children and, if unrecognized, can serve as a source of nosocomial tinea corporis for hospital staff.<sup>98</sup> The manifestations of tinea capitis vary and include non-inflammatory and inflammatory variants. Oral griseofulvin plus selenium sulfide shampoos is the traditional treatment of choice for tinea capitis and kerion.<sup>96</sup> More recent alternatives include systemic therapy with fluconazole, itraconazole or terbinafine.<sup>99</sup> These approaches may be particularly useful in immunocompromised patients who may fail conventional therapy or who have locally invasive infections extending into the dermis and causing painful erythematous, nodular or ulcerative lesions.<sup>100</sup>

*Malassezia furfur* and *Malassezia pachydermatis* are the agents of tinea versicolor that presents with hypopigmented macules on the upper trunk. Application of long-wave UV by a Wood's lamp aids in the clinical diagnosis, and skin scrapings reveal typical clusters of blastoconidia and hyphae in the classic 'spaghetti and meatballs' pattern. Treatment is accomplished with selenium sulfide shampoo or topical agents; newer alternatives include oral itraconazole or fluconazole.

*Candida albicans* is a ubiquitous agent of diaper dermatitis that may be precipitated by moisture, occlusion, fecal contact and urinary pH. Its classic presentation is that of an erythema bordered by a collarette of scale with satellite papules and pustules. Concomitant dermatophytosis may occasionally be present. Treatment consists of the correction of physiologic factors and topical antifungal treatment.<sup>16</sup>

## Diagnostic considerations

Effective management of invasive fungal infections requires early and accurate diagnosis. Microscopy and culture of appropriate specimens remain the gold standard of mycologic diagnosis. However, culture is followed by many problems such as difficulty to obtain an appropriate and/or sufficient specimen, long duration of culturing and negative results. Histology and microscopic examination are fast techniques, but they are either performed very late or they are not sensitive enough. In addition, in neonates and infants they are almost impossible to perform.<sup>42</sup>

High-resolution computed tomography, serially performed, constitutes a sensitive mode of diagnosis of pulmonary mould infections in hematologic patients, but similar data are scarce for children. Three studies have examined the role of radiology in the diagnosis of invasive aspergillosis in pediatric patients. In the most recent study, 20 patients with a mean age of 5 years (7 months to 18 years) with invasive pulmonary aspergillosis had radiographic findings that included segmental

and lobar consolidation, perihilar infiltrates, multiple small nodules, peripheral nodular masses and pleural effusions.<sup>101</sup> In general, pulmonary findings were varied and often non-specific. No patients with invasive pulmonary aspergillosis exhibited a “halo” sign. This difference may be due to differences in the underlying pathology of invasive pulmonary aspergillosis in non-hematologic pediatric patients, such as non-angioinvasive disease seen in CGD, differences in underlying disease as well as the particular age-dependent immune response of the host. In contrast to adult disease, the incidence of central cavitation of pulmonary lesions appeared to be low (25%). No evidence of air crescent formation within areas of consolidation on CT existed, as compared to approximately 50% with cavitation and 40% with air crescent formation in adult series.<sup>101</sup> In the other two pediatric reports, 22% of patients with cavitation on chest x-ray and 43% with cavitation on CT were reported, respectively.<sup>42</sup>

Early diagnosis of invasive pulmonary aspergillosis has been improved with galactomannan ELISA assay in adults. Several studies in older patients have shown that galactomannan assay has an overall sensitivity of >85%, ranging from 60% to 92.6%, and a specificity ranging above 85%.<sup>102</sup> However, its use is problematic in pediatric patients, and especially in young infants, due to specificity or sensitivity inferiority. In one prospective study, 450 adult allogeneic hematopoietic stem cell transplant (HSCT) patients (3883 samples) as well as 347 children with hematologic malignancies (2376 samples) were followed.<sup>103</sup> In this study, the false-positive rate in adult patients was 2.5% as compared to 10.1% in children. In another study, the false-positive rate in a group of neutropenic patients with 797 episodes of fever of unknown origin, including 48 pediatric patients, was 0.9% in adults and 44% in children ( $P<0.0001$ ).<sup>104</sup> Overall positive predictive value was calculated to be 92.1% for adult HSCT recipients and 15.4% for children ( $P<0.0001$ ). Similarly to the observed lack of the “halo” sign in pediatric patients, this may be due to differences in the particular pathology of invasive pulmonary aspergillosis in the two patient populations, differences in underlying disease as well as age-dependent immune response of the host to the infection.

Antibiotics, primarily piperacillin-tazobactam but also amoxicillin alone or combined with clavulanic acid, represent a source of extraneous galactomannan that may compromise clinical specificity of the assay.<sup>105</sup> This may be due to the presence of galactomannan in the drugs themselves, leading to false-positive results and thus to an inherent difficulty in using the test for patients receiving these antibiotics.

An important age-dependent problem of utility of galactomannan assay in pediatric patients is the low specificity of the assay observed in neonates and young infants.<sup>106</sup> Lipoteichoic acid, a constituent of bacterial walls of *Bifidobacterium bifidum* subspecies *pennsylvanicum*, abundantly existing in the intestinal microflora of neonates, may mimic the epitope recognized by the EB-A2 in the ELISA galactomannan kit.<sup>106</sup> In addition, galactomannan-positive infant formula used in neonates as well as galactomannan-containing food and even water, combined with increased translocation across an immature or damaged intestinal epithelium, may be responsible for the false positivity observed in the neonates.

One study evaluated patients with CGD or Job’s syndrome who suffered from invasive aspergillosis and found

that galactomannan was detected in only four of 15 (27%) cases of CGD and Job’s syndrome as compared to 24 of 30 (80%) cases of all other immunocompromising conditions ( $P=0.0004$ ).<sup>107</sup> The reasons for this difference may be more localized and less angioinvasive disease in CGD patients, particular sites of infection, i.e., bone infections that are more frequent in these patients than in hematologic patients, or certain immune factors unique in CGD patients. For example, these patients possess intact T and B lymphocytic functions as well as normal numbers of phagocytes, whereas patients with hematologic malignancies or HSCT may have profoundly impaired lymphocyte number and/or function as well as non-existent phagocytes.

$\beta$ -D-glucan assay has been studied in adult patients with fungal infections.<sup>108</sup> Particularly in neonates, the assay is very promising for the diagnosis of invasive candidiasis, which is the most frequent fungal infection in this patient population. By excluding invasive candidiasis in high-risk patients with this assay, therapy can be delayed or withheld by physicians, whilst early, preemptive or targeted antifungal therapy can be initiated in those patients who show positive results.

The polymerase chain reaction (PCR) may be a powerful tool for early diagnosis of invasive fungal infections but has not yet been standardized for routine use. No studies address the issue in neonates, whereas in children PCR has not been specifically studied but is probably as good as in adults. A high degree of suspicion in immunodeficient pediatric hosts, suggestive clinical and radiologic findings as well as mycologic data by application of multiple diagnostic methods, including serology and molecular biology, can enhance the capacity to diagnose invasive fungal infections in young patients.

## Pediatric pharmacology of established antifungal agents

The general pharmacology of antifungal agents has been reviewed in other chapters of this textbook. The following sections summarize relevant data on safety, pharmacokinetics and dosing of antifungal agents in pediatric patients, current indications and approval status. A more detailed review of the pediatric pharmacology of antifungal agents can be found elsewhere.<sup>109</sup>

### Amphotericin B deoxycholate

The pharmacokinetics of amphotericin B deoxycholate (D-AmB) in pediatric patients is characterized by a high inter-individual variability.<sup>110</sup> Infants and children appear to clear the drug more rapidly than adults. However, whether this enhanced clearance from the bloodstream has implications for dosing is unknown.

Infusion-related reactions and nephrotoxicity often limit therapy with D-AmB. In a prospective study in pediatric cancer patients, fever and/or rigors were observed in 19 of 78 treatment courses (24%).<sup>111</sup> In the neonatal setting, however, infusion-related reactions are only rarely observed.<sup>112</sup> Infusion-related reactions may be blunted by slowing the infusion rate but often require acetaminophen, hydrocortisone (0.5–1.0 mg/kg) or meperidine (0.2–0.5 mg/kg) premedication.<sup>16</sup> While some data suggest a somewhat lower rate of azotemia in children as



compared to adults,<sup>113</sup> this has not been a consistent observation. D-AmB associated azotemia has been reported in only 2% of pediatric cancer patients receiving the drug at 1 mg/kg/day for comparatively short periods as empiric antifungal therapy;<sup>110</sup> in premature neonates, the incidence of azotemia ranges from zero to 15% in more contemporary studies,<sup>112,114-116</sup> indicating that D-AmB is much better tolerated, as reported early during its use.<sup>117</sup> Avoiding concomitant nephrotoxic agents and using appropriate hydration and normal saline loading (10–15 ml NaCl/kg/day)<sup>118-120</sup> may lessen the likelihood and severity of azotemia.

Few indications are left for antifungal treatment of opportunistic mycoses with D-AmB. These include candidemia and acute disseminated candidiasis, particular in neonates, and induction therapy for cryptococcal meningitis. The recommended daily dosage in these settings ranges from 0.7 to 1.0 mg/kg/day administered over 2–4 hours as tolerated. Treatment should be started at the full target dosage with careful monitoring during the first hour of infusion.

### Lipid formulations of amphotericin B

The lipid formulations of amphotericin B (amphotericin B colloidal dispersion (ABCD; Amphocil™ or Amphotec™), amphotericin B lipid complex (ABLC; Abelcet™), and a small unilamellar vesicle (SUV) liposomal formulation (L-AmB; AmBisome™) share a reduced nephrotoxicity, which allows for the safe delivery of higher dosages of AmB.<sup>121</sup> Each of the lipid formulations possesses distinct physicochemical and pharmacokinetic properties (Table 22-3). Whether and how the distinct physicochemical and pharmacokinetic features translate into different pharmacodynamics in vivo is largely unknown.

#### Amphotericin B Colloidal Dispersion (ABCD)

Compartmental pharmacokinetic analyses of a few immunocompromised children <13 years who received the compound at 7.0 and 7.5 mg/kg/day revealed no fundamental differences in the plasma pharmacokinetics as compared to a dose-matched cohort of adult patients.<sup>122</sup> Safety data of approximately 100 pediatric patients enrolled in a prospective, randomized trial comparing ABCD with D-AmB and in five different open-label phase 2 trials of ABCD showed significantly less renal toxicity in children receiving ABCD than in those receiving D-AmB (12.0% vs 52.4%;  $P=0.003$ ); other adverse symptoms were not significantly different. In the additional open-label studies, although 80% of patients receiving ABCD reported some adverse symptoms, the majority of these were infusion related, and nephrotoxicity was reported in only 12%; there were no other unexpected severe toxicities.<sup>123</sup>

#### Amphotericin B Lipid Complex (ABLC)

Pharmacokinetic studies of ABLC in pediatric cancer patients who received the compound at 2.5 mg/kg over 6 weeks for hepatosplenic candidiasis have demonstrated that no differences were observed as compared to adults.<sup>124</sup>

In a larger phase 2 salvage study including 111 treatment episodes in pediatric patients, the mean serum creatinine for the entire study population did not change between baseline ( $1.23 \pm 0.11$  mg/dl) and end of therapy ( $1.32 \pm 0.12$  mg/dl) during 6 weeks. Similarly, no significant differences were observed for serum potassium, magnesium, hepatic transaminases, alkaline phosphatase, and hemoglobin. However, there was an increase in the mean total bilirubin ( $3.66 \pm 0.73$  to  $5.13 \pm 1.09$  mg/dl) at the end of therapy ( $P=0.054$ ). In seven patients (6%), ABLC therapy was discontinued because of one or more adverse effects.<sup>125</sup> In 548 children and adolescents who were enrolled in

**Table 22-3** Physicochemical properties and multiple-dose pharmacokinetic parameters of the four licensed amphotericin B formulations

	D-AmB	ABCD	ABLC	L-AmB
Lipids (molar ratio)	Deoxycholate	Cholesteryl sulfate	DMPC/DMPG (7:3)	HPC/CHOL/DSPG (2:1:0.8)
Mol% AmB	34%	50%	50%	10%
Lipid configuration	Micelles	Micelles	Membrane-like	SUVs
Diameter (μm)	0.05	0.12–0.14	1.6–11	0.08
Dosage (mg AmB/kg)	1	5	5	5
C <sub>max</sub> (μg/ml)	2.9	3.1	1.7	58
AUC <sub>0-∞</sub> (μg/ml/h)	36	43	14	713
VD <sub>ss</sub> (l/kg)	1.1	4.3	131	0.22
Cl (l/h/kg)	0.028	0.117	0.476	0.017

HPC, hydrogenated phosphatidylcholine; CHOL, cholesterol; DSPG, distearyl phosphatidylglycerol; DMPC, dimiristoyl phosphatidylcholine; DMPG, dimiristoyl phosphatidylglycerol; SUV, small unilamellar vesicles; C<sub>max</sub>, peak plasma concentration; AUC<sub>0-∞</sub>, area under the concentration vs time curve from time zero to infinity; VD<sub>ss</sub>, apparent volume of distribution at steady state; Cl, plasma clearance. Data represent mean values, stem from adult patients and were obtained after different rates of infusion (modified from Groll et al 1998<sup>121</sup>).

the Collaborative Exchange of Antifungal Research (CLEAR) registry of the manufacturer, elevations in serum creatinine of  $>1.5\times$  baseline and  $>2.5\times$  baseline values were seen in 24.8% and 8.8% of all patients, respectively.<sup>126</sup>

A population pharmacokinetic study in 28 mostly immature neonates with invasive *Candida* infections has demonstrated that the disposition of ABLC in neonates is similar to that observed in other age groups; weight was the only factor that influenced clearance.<sup>127</sup>

#### Liposomal Amphotericin B (L-AmB)

Two pharmacokinetic studies in pediatric patients indicate that the disposition of L-AmB is not fundamentally different from that in adults and that weight is a covariate that determines clearance and volume of distribution.<sup>128</sup> Safety data are available from 204 children with neutropenia and fever who were randomized in an open-label, multicenter comparative trial. Twenty-nine percent of patients treated with L-AmB 1 mg/kg, 39% of patients treated with L-AmB 3 mg/kg, and 54% of patients treated with D-AmB 1 mg/kg experienced adverse effects ( $P=0.01$ ); nephrotoxicity, defined as 100% or more increase in serum creatinine from baseline, was noted in 8%, 11%, and 21%, respectively (n.s.). Hypokalemia ( $<2.5$  mmol/l) occurred in 10%, 11%, and 26% of patients ( $P=0.02$ ), increases in serum transaminase levels ( $\geq 110$  U/l) in 17%, 23%, and 17%, and increases in serum bilirubin ( $\geq 35\mu\text{mol/l}$ ) in 11%, 12%, and 10% of patients, respectively.<sup>113</sup> A phase 4 analysis of 141 courses of L-AmB administered for a mean of 17 days' duration at a mean maximum dosage of 2.5 mg/kg for various indications to pediatric cancer/HSCT patients revealed a low rate of adverse events (4%) necessitating discontinuation.<sup>129</sup>

L-AmB (2.5–7 mg/kg/day) was evaluated prospectively in 24 VLBW infants (mean birth weight 847 $\pm$ 244 g, mean gestational age 26 weeks) with mostly refractory systemic candidiasis. The mean duration of therapy was 21 days. Twenty (83%) infants were considered clinically cured at the end of treatment. No major adverse effects were recorded; one infant developed increased bilirubin and hepatic transaminases levels during therapy. Four (17%) infants died; in two of them (8%) the cause of death was directly attributed to systemic candidiasis.<sup>130</sup> High-dose (5–7 mg/kg/day) L-AmB was evaluated prospectively in 41 episodes of systemic candidiasis occurring in 37 premature neonates. The median duration of therapy was 18 days. Fungal eradication was achieved in 39 of 41 (95%) episodes; one patient died due to systemic candidiasis on day 12 of therapy. Fungal eradication was more rapid in patients treated early with high doses and in patients who received high-dose L-AmB as first-line therapy.<sup>130</sup>

The lipid formulations of amphotericin B are currently licensed for the treatment of patients with invasive mycoses refractory to or intolerant of D-AmB, and, limited to L-AmB, for empiric therapy of persistently neutropenic patients. Evidence-based, but currently not licensed, indications for first-line therapy exist for L-AmB for treatment of invasive aspergillosis,<sup>131</sup> invasive candidiasis,<sup>132</sup> and zygomycosis (all formulations).<sup>133</sup> The recommended therapeutic dosages are 3–5 mg/kg/day for L-AmB, and 5 mg/kg for ABCD and ABLC, respectively;<sup>133</sup> the therapeutic dosage for treatment of zygomycosis should not be less than 5 mg/kg/day. Similar to D-AmB, treatment should be started with the calculated full dosage at the infusion rate recommended by the manufacturer.

## Antifungal triazoles

The antifungal triazoles have become an important component of the antifungal armamentarium. Whereas fluconazole and itraconazole are available for more than a decade, new triazoles such as voriconazole and posaconazole have entered the clinical arena only recently.<sup>134,135</sup>

### Fluconazole

With the exception of premature neonates, in whom clearance is decreased, pediatric patients tend to have an increased normalized plasma clearance and a shorter half-life in comparison to adults<sup>136–141</sup> (Table 22-4). As a consequence, dosages at the higher end of the recommended dosage range are necessary for the treatment of invasive mycoses in children.

In pediatric patients of all age groups, at dosages of up to 12 mg/kg/day, fluconazole is generally well tolerated.<sup>142</sup> The most common reported side effects in pediatric patients include gastrointestinal disturbances (8%), increases in hepatic transaminases (5%) and skin reactions (1%); toxicity-related discontinuation of therapy with fluconazole occurs in approximately 3% of patients.<sup>142</sup> Severe side effects, including relevant hepatotoxicity and exfoliative skin reactions, have been reported in association with fluconazole therapy.<sup>134</sup>

Apart from treatment of oropharyngeal and esophageal candidiasis, fluconazole may be used for invasive *Candida* infections caused by susceptible organisms in patients who are in a stable condition and who have not received prior azole therapy.<sup>143</sup> This also applies to the neonatal setting. In six published series including  $\geq 10$  patients with proven invasive *Candida* infections, treatment with fluconazole at a daily dosage of 5–6 mg/kg was successful in 83–97% and crude mortality ranged from 10% to 33%; in none of the altogether 125 patients was fluconazole discontinued due to toxicity.<sup>144–149</sup> The recommended dosage range for pediatric patients of all age groups is 6–12 mg/kg/day; in view of the faster clearance

**Table 22-4** Pharmacokinetic parameters of fluconazole in pediatric patients

Age group	Vd <sub>ss</sub> (l/kg)	Cl (l/h/kg)	T <sub>1/2</sub> $\beta$ (h)
Preterm <1500 g: day 1	1.18	0.010	88
day 6	1.84	0.019	67
day 12	2.25	0.031	55
Term neonates	1.43	0.036	28
Infants >1–6 months	1.02	0.037	19
Children, 5–15 years	0.84	0.031	18
Adult volunteers	0.65	0.015	30
Data represent mean values and are compiled from six studies. <sup>136–141</sup> Vd <sub>ss</sub> , apparent volume of distribution at steady state; Cl, total plasma clearance; t <sub>1/2</sub> $\beta$ , elimination half-life.			

rate, however, 12 mg/kg/day may be the most appropriate dosage for treatment of life-threatening infections. Because of an initially decreased clearance in preterm neonates of <1500 g, we advocate every other day dosing with 6–12 mg/kg during the first week of life in this specific setting.

Further potential therapeutic and prophylactic indications for fluconazole are similar to those reviewed in other chapters of this textbook. In LBW infants, fluconazole has been shown to reduce *Candida* infections;<sup>150</sup> based on these studies, fluconazole prophylaxis is a valid option for centers with a high frequency (>10%) of invasive *Candida* infections in premature infants of <1000 g birth weight or in the setting of a nosocomial outbreak by a fluconazole-susceptible *Candida* species.

#### Itraconazole

The safety and pharmacokinetics of cyclodextrin itraconazole solution in immunocompromised pediatric patients have been studied in two phase 2 clinical trials.<sup>151,152</sup> The solution was well tolerated and safe in 26 infants and children with cancer (n = 20) or liver transplantation who received the compound at 5 mg/kg qd for documented mucosal candidiasis or as antifungal prophylaxis for 2 weeks. Treatment with cyclodextrin itraconazole achieved potentially therapeutic concentrations of itraconazole in plasma; these levels, however, were substantially lower than those reported in adult cancer patients<sup>153</sup>

(Table 22-5). In a cohort of 26 HIV-infected children and adolescents, cyclodextrin itraconazole was safe and effective for treatment of oropharyngeal candidiasis at dosages of 2.5 mg qd or 2.5 mg bid given for at least 14 days. Both dosage regimens resulted in higher peak plasma concentrations and AUC<sub>0–24 hr</sub> values than reported in the above referenced study in pediatric cancer patients. Based on safety and efficacy, a dosage of 2.5 mg/kg bid was recommended for the treatment of OPC in pediatric patients ≤5 years old.<sup>152</sup> Vomiting (12%), abnormal liver function tests (5%) and abdominal pain (3%) were the most common adverse effects considered definitely or possibly related to cyclodextrin itraconazole solution in an open study in 103 neutropenic pediatric patients who received the drug at 5mg/kg/day for antifungal prophylaxis; 18% of patients withdrew from the study because of adverse events.<sup>154</sup> Pharmacokinetics and safety of the intravenous formulation in pediatric patients have not been reported yet; similarly, only anecdotal reports have been published on the use of itraconazole in the neonatal setting.

Therapeutic and prophylactic indications of itraconazole in the settings of superficial and invasive mycoses have been reviewed in other chapters of this textbook. Of note, the compound is not approved for use in pediatric age groups <18 years of age and can therefore only be used off label. Based on published pharmacokinetic and safety data, the starting dosage range for oral itraconazole in pediatric patients beyond

**Table 22-5** Pharmacokinetics of itraconazole in immunocompromised children following administration of the oral hydroxypropyl-β-cyclodextrin solution

	Children with Cancer/Liver Tx (n = 8, 0.5–2 yr) 5.0 mg/kg qd × 14 days	Children with Cancer (n = 7, 2–5 yr) 5.0 mg/kg qd × 14 days	Children with Cancer (n = 11, 5–12 yr) 5.0 mg/kg qd × 14 days
<b>Itraconazole</b>			
C <sub>max</sub> (μg/ml)	0.571±0.416	0.534±0.431	0.631±0.358
T <sub>max</sub> (h)	1.9±0.1	2.9±2.5	3.1±2.1
AUC <sub>0–24</sub> (μg/ml/h)	6.930±5.83	7.33±5.42	8.77±5.05
T <sub>1/2β</sub> (h)	47.4±55.0	30.6±25.3	28.3±9.6
Acc.factor	6.2±5.0	3.3±3.0	8.6±7.4
<b>OH-Itraconazole</b>			
C <sub>max</sub> (μg/ml)	0.690±0.445	0.687±0.419	0.699±0.234
T <sub>max</sub> (h)	4.4±2.3	4.8±2.7	10.8±14.3
AUC <sub>0–24</sub> (μg/ml/h)	13.20±11.40	13.4±9.1	13.45±7.19
T <sub>1/2β</sub> (h)	18.0±18.1	17.1±14.5	17.9±8.7
Acc.factor	11.4±16.0	2.3±1.9	6.4±5.6
All values represent mean values ± SD; modified from de Repentigny. <sup>151</sup> C <sub>max</sub> , peak plasma levels; T <sub>max</sub> , time until occurrence of C <sub>max</sub> ; AUC <sub>0–24</sub> , area under the concentration vs time curve from 0 to 24 hours; T <sub>1/2β</sub> , elimination half-life; accumulation factor (AUC <sub>0–24</sub> day 14/AUC <sub>0–24</sub> day 1).			



the neonatal period is 5 mg/kg/day in two divided doses of the oral suspension. The recommended target level is  $>0.5$   $\mu\text{g/ml}$  of the parent itraconazole before the next dose, as measured by high performance liquid chromatography (HPLC).<sup>133</sup> Data on the use of intravenous itraconazole in pediatric patients are currently lacking; the dosage regimen utilized in the published adult studies is 200 mg bid for 2 days, followed by 200 mg qd for a maximum of 12 days.<sup>155,156</sup>

## 5-Fluorocytosine (5FC)

5-Fluorocytosine (5FC) is a fungus-specific synthetic base-analog that acts by causing RNA miscoding and inhibition of DNA synthesis. In the US, 5FC is available only as oral formulation; in several European countries, it is also marketed as intravenous solution. Whereas separate pharmacokinetic data for infants and children are lacking, an extreme interindividual variability in clearance and distribution volume has been reported in neonates.<sup>157</sup>

An established indication for 5FC is its use in combination with D-AmB for induction therapy of cryptococcal meningitis.<sup>158,159</sup> The combination with D-AmB may also be recommended for the treatment of *Candida* infections involving deep tissues, in particular for *Candida* meningitis, and severe infections by *C. glabrata*.<sup>16</sup>

Close monitoring of plasma levels and adjustment of the dosage is recommended, in particular when there is evidence for impaired renal function; peak plasma levels between 40 and 60  $\mu\text{g/ml}$  correlate with antifungal activity but are seldom associated with marrow toxicity.<sup>160</sup> A starting dosage for both adults and children of 100 mg/kg daily divided in 3–4 doses is currently recommended.

## New agents for treatment and prevention and their pediatric development

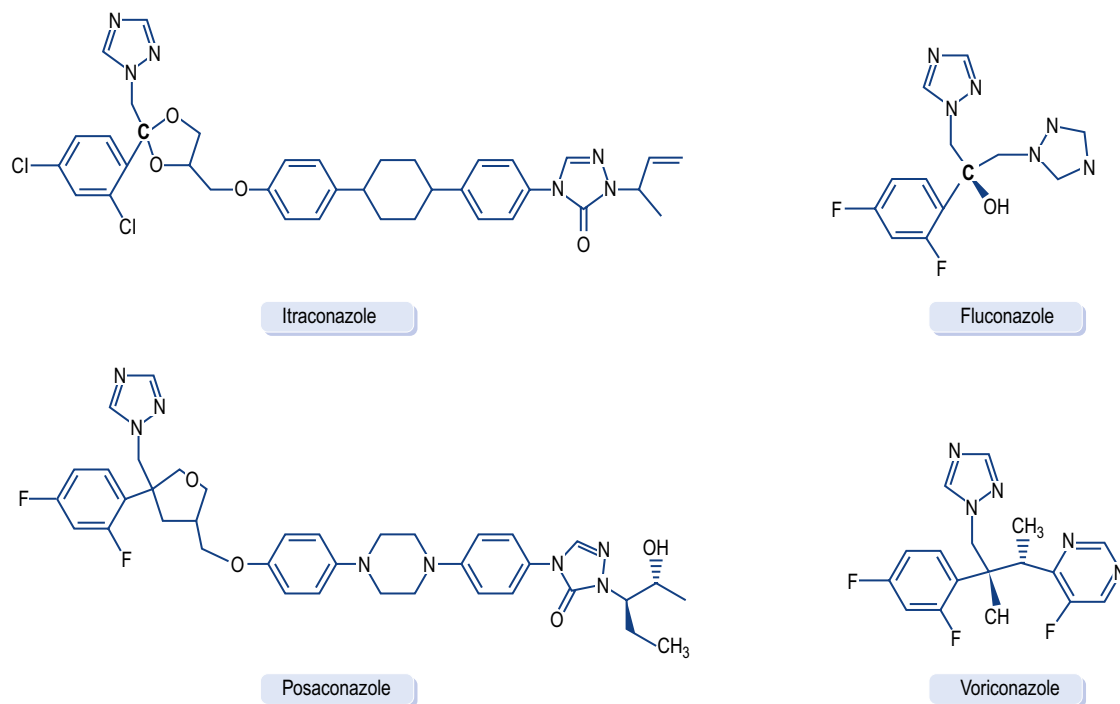
### New antifungal triazoles

The structures of the new triazoles and, for comparison, those of fluconazole and itraconazole are listed in Figure 22-1.

#### Voriconazole

Voriconazole is a synthetic oral and parenteral antifungal triazole with activity against a wide spectrum of clinically important yeasts and moulds. Based on clinical phase 2 and 3 trials, voriconazole is currently approved for treatment of invasive aspergillosis, fusariosis and scedosporiosis, and for primary treatment of invasive candidiasis in non-neutropenic patients. The recommended IV dosages for patients 12 years and above are 6 mg/kg bid on day 1, followed by 4 mg/kg bid. The oral dosages in adults are 400 mg bid on day 1 ( $<40$  kg: 200 mg bid), followed by 200 mg bid ( $<40$  kg: 100 mg bid).

Pediatric patients  $<12$  years of age have a higher capacity for elimination of voriconazole per kilogram of body weight than adult healthy volunteers, resulting in a lower, potentially non-therapeutic exposure at similar dosages<sup>161</sup> (Table 22-6). An intraindividual dosage escalation study exploring pharmacokinetics and safety of higher dosage regimens of voriconazole in this patient population has been completed; based on the population-based analysis of the dataset of that study, an IV dosage of 7 mg/kg bid and an oral dosage of 200 mg bid (oral suspension) without loading dosages is currently recommended for children  $>1$ –11 years of age.<sup>162</sup>



**Figure 22-1** Chemical structures of voriconazole and posaconazole in comparison to fluconazole and itraconazole. Whereas voriconazole is structurally related to fluconazole, posaconazole is structurally related to itraconazole.

**Table 22-6** Simulated plasma concentrations of voriconazole following multiple doses of 3 and 4 mg/kg in pediatric and adult patients

Parameter	Value			
	Pediatric Patients 2–11 yr		Adult Patients	
	3 mg/kg	4 mg/kg	3 mg/kg	4 mg/kg
AUC <sub>tau</sub> (ng * h/ml)	10,670	14,227	13,855	38,605
C <sub>average</sub> (ng/ml)	889	1,186	1,155	3,217

Data are reported as medians following 6 mg/kg 12 every h on day 1 and maintenance dose 3. While in pediatric patients, an increase in dosage by a factor of 1.3 leads to proportional increase in the mean average plasma concentration (C<sub>average</sub>) and in the mean area under the concentration-vs-time curve (AUC<sub>tau</sub>), adult patients display a 2.8-fold, hyperproportional increase in exposure, indicating non-linear disposition. As a consequence, pediatric patients dosed at 4 mg/kg do not achieve the same exposure as adults dosed at 4 mg/kg, the dosage that has led to the approval of voriconazole for first-line treatment of invasive aspergillosis (modified from Walsh et al<sup>162</sup>).

Voriconazole has been administered safely and with success to a number of children <12 years without therapeutic alternative. Of 58 immunocompromised children with proven or probable invasive fungal infection, 26 (45%) had a complete or partial response. Four (7%) were discontinued because of intolerance. A total of 23 patients had voriconazole-related adverse events, most commonly elevation in hepatic transaminases or bilirubin (n = 8), skin rash (n = 8), abnormal vision (n = 3) and photosensitivity reactions (n = 3).<sup>163</sup> The safety and tolerance of voriconazole were also analyzed in a retrospective cohort study in 37 immunocompromised children and adolescents who received the drug at dosages ranging from 2 to 8 mg/kg twice daily for a mean duration of 174 days (range, 5–998 days). Grade I or II adverse events were observed in 19 patients (51%); the most frequent events included transient increases in hepatic transaminases<sup>19</sup> and transient visual disturbances.<sup>5</sup> Four patients (10%) experienced grade III/IV adverse events and three (8%) were permanently discontinued. While not a primary endpoint of the analysis, voriconazole showed promising efficacy as a preventive and therapeutic modality.<sup>164</sup>

#### Posaconazole

Posaconazole is a novel oral antifungal triazole with potent and broad-spectrum activity against opportunistic, endemic, and dermatophytic fungi *in vitro*. Importantly, posaconazole also possesses activity against zygomycetes both *in vitro* and *in vivo*, distinguishing it from all available azoles.<sup>165</sup>

Posaconazole has been approved in the European Union in patients ≥18 years for treatment of aspergillosis, fusariosis, chromoblastomycosis and coccidioidomycosis refractory to or intolerant of standard therapies. In addition, it is approved for prophylaxis in high-risk patients with acute myeloid leukemia/myelodysplastic syndrome (AML/MDS), HSCT and graft-versus-host disease (GvHD) in both the European Union and the United States. The recommended daily dosage for salvage treatment is 400 mg bid given with food; for patients not tolerating solid food, a dosage of 200 mg qid is recommended, preferentially together with a nutritional supplement. The dosage for prophylaxis is 200 mg tid.<sup>165</sup>

The pharmacokinetics of posaconazole in pediatric patients (<18 years of age) has not been studied yet. Very limited data obtained in 12 pediatric subjects ≥8 years of age appear

to indicate no fundamental differences in trough plasma concentrations as compared to adults.<sup>166</sup> Salvage treatment with posaconazole resulted in successful outcomes in five of 11 pediatric subjects (8–17 years of age), which appears similar to the outcome in the adult population.<sup>167</sup>

A larger number of pediatric patients have received the compound within the manufacturer's compassionate use program without signal for differences in safety as compared to adult patients. A pediatric development program has been initiated to define dosages, safety and tolerance in the pediatric population beyond the neonatal period.

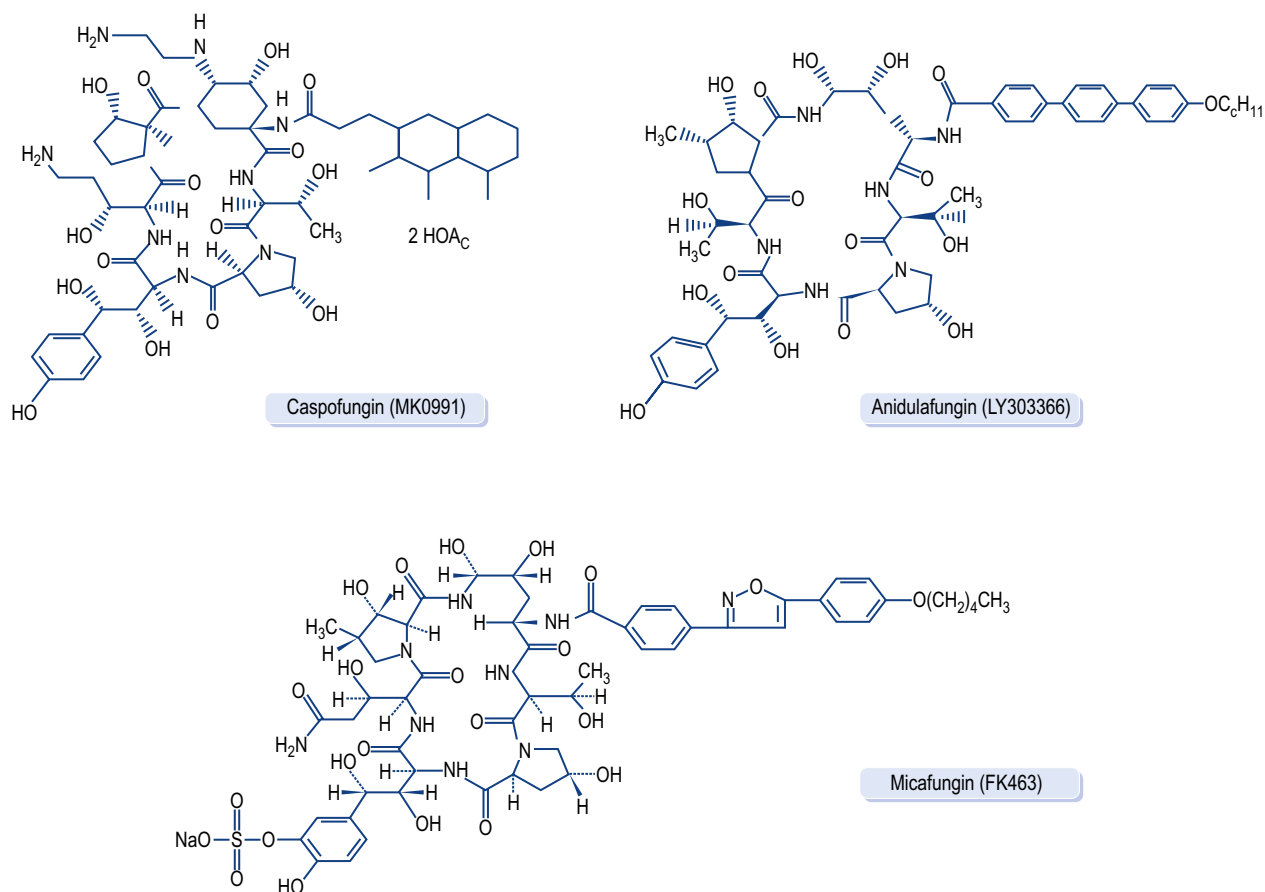
#### Echinocandin lipopeptides

The echinocandins are a distinct class of intravenous semi-synthetic amphiphilic lipopeptides that act by inhibiting the synthesis of 1,3-β-D-glucan in the fungal cell wall. Over the past decade, three compounds with similar spectrum, pharmacokinetics, safety and antifungal efficacy have been developed: anidulafungin (Eraxis™), caspofungin (Cancidas™), and micafungin (Mycamine™) (Fig. 22-2).

#### Caspofungin

Caspofungin was the first licensed compound of the echinocandin class of antifungal agents. It is licensed in the European Union and the United States in patients ≥18 years for second-line therapy of definite or probable invasive aspergillosis, for primary therapy in non-neutropenic patients with invasive *Candida* infections, and for empiric antifungal therapy in granulocytopenic patients with persistent fever. The recommended dose regimen consists of a single 70 mg loading dose on day 1, followed by 50 mg daily thereafter, administered over 1 hour.<sup>168</sup>

In children and adolescents, the pharmacokinetics and safety of caspofungin were investigated using either a weight-based regimen (1 mg/kg of body weight/day) or a body surface area regimen (50 mg/m<sup>2</sup>/day). Compared to adult patients treated with 50 mg/day, the maintenance dosage of 1 mg/kg/day achieved suboptimal exposure, whereas a maintenance dosage of 50 mg/m<sup>2</sup>/day provided similar or slightly higher exposure relative to adults<sup>169</sup> (Table 22-7). As a consequence, a dosage of 50 mg/m<sup>2</sup>/day (day 1, 70 mg/m<sup>2</sup>/day; maximum daily dose, 70 mg) has been selected for the further pediatric



**Figure 22-2** Chemical structures of caspofungin, anidulafungin, and micafungin. The echinocandins are amphoteric cyclic lipopeptides with a hexapeptide core that is linked to a variably configured lipid side chain.

**Table 22-7** Single-dose caspofungin pharmacokinetics in pediatric and adult patients

Dosage	Children (2–11 yr)		Adolescents (12–17 yr) 50 mg/m <sup>2</sup>	Adults 50 mg
	1 mg/kg	50 mg/m <sup>2</sup>		
AUC <sub>0–24h</sub> (μg × h/ml)	41.5	96.4	77.6	70.6
C1 (μg/ml)	6.59	13.9	7.67	7.67
C24 (μg/ml)	0.45	1.09	1.35	1.35
T <sub>1/2 β</sub> (h)	7.2	7.6	11.7	11.7
CL (ml/min/m <sup>2</sup> )	8.57	7.78	6.07	6.07

Least square means are reported for AUC, C1 (C<sub>max</sub>), and C24 (C<sub>min</sub>), and harmonic means for T<sub>1/2 β</sub>. Data were obtained in groups of 6–10 pediatric patients and compared to those obtained in 32 adult patients with mucosal candidiasis. In comparison to adult values, a 1 mg/kg dosage does not achieve the target trough concentration of caspofungin of 1 μg/ml and leads to a lower exposure as measured by the AUC<sub>0–24h</sub>; the dosage regimen of 50 mg/m<sup>2</sup> leads to similar or slightly higher trough concentrations and similar or slightly higher exposure (AUC<sub>0–24h</sub>) and has been selected for the further development program in pediatric patients (modified from Walsh et al<sup>169</sup>).

program. Although not yet approved in this population, caspofungin appears to be well tolerated in pediatric patients. In the above-mentioned phase 1/2 dose-finding study in 39 children and adolescents, none of the patients developed a serious drug-related adverse event or was discontinued for toxicity.<sup>169</sup>

A similarly favorable safety profile has also been reported in immunocompromised pediatric patients who received the compound for various indications mostly in combination with other antifungal agents<sup>170,171</sup> and in neonates with refractory invasive candidiasis.<sup>172–174</sup>



**Table 22-8** Single-dose anidulafungin pharmacokinetics in pediatric and adult patients

Dosage	Pediatric Patients (2–17 yr)		Adults	
	0.75 mg/kg	1.5 mg/kg	50 mg	100 mg
C <sub>max</sub> (μg/ml)	4.02	6.09	2.51	3.82
AUC <sub>0–24h</sub> (μg × h/ml)	48.0	89.7	53.3	104.8
T <sub>1/2 β</sub> (h)	20.8	19.5	39.3*	42.3*
CL (l/h/kg)	0.0175	0.0191	n/a	n/a
VD <sub>ss</sub> (l/kg)	0.45	0.49	0.72	0.78

Data were obtained in groups of six pediatric patients with compromised immunity and neutropenia per age group and dosage level, and compared to those obtained in 26 adult healthy volunteers. Modified from Benjamin et al.<sup>175</sup> and Chiou et al.<sup>109</sup> n/a, not available.

\*T<sub>1/2 β</sub>

**Table 22-9** Single-dose micafungin pharmacokinetics in pediatric and adult patients

Dosage	Pediatric Patients (2–17 yr)			Adults	
	1 mg/kg	2 mg/kg	4 mg	50 mg	100 mg
C <sub>max</sub> (μg/ml)	10.8	15.3	30.3	3.6	7.1
AUC <sub>0–24h</sub> (μg × h/ml)	40.3	83.0	191.4	33.9	59.9
T <sub>1/2 β</sub> (h)	12.5	13.2	11.6	12.5	13.0
CL (l/h/kg)	0.021	0.020	0.017	0.017*	0.018*
VD <sub>ss</sub> (l/kg)	0.33	0.31	0.28	0.31*	0.32*

Pharmacokinetic parameters are expressed as mean values. Data were obtained in groups of 7–15 pediatric patients with compromised immunity and neutropenia per age group and dosage level, and compared to those obtained in cohorts of 8–9 adult patients with hematopoietic stem cell transplantation. Modified from Seibel et al.<sup>177</sup> and Hebert et al.<sup>176</sup>

\*Weight normalization calculated by assuming an average body weight of 70 kg.

### Anidulafungin

Anidulafungin is licensed in the United States in patients ≥18 years of age for primary therapy in non-neutropenic patients with invasive *Candida* infections and for esophageal candidiasis. Regulatory approval in Europe is expected in the near future. The recommended dose regimen consists of 100 mg (day 1, 200 mg) for invasive candidiasis and 50 mg qd (day 1, 100mg) for esophageal candidiasis, administered at a rate not exceeding 1.1 mg/minute.

A pediatric phase 1/2 multicenter study of the pharmacokinetics and safety of anidulafungin has been completed in 19 granulocytopenic children with cancer. Patients were divided into two age cohorts (2–11 and 12–17 years) and were enrolled into sequential groups to receive 0.75 or 1.5 mg/kg/day<sup>175</sup> (Table 22-8). No drug-related serious adverse events were recorded. Pharmacokinetic parameters were similar across age groups and dosage cohorts and similar relative to adult subjects. Following single and multiple daily doses of 0.75 mg/kg and 1.5 mg/kg, plasma concentration data corresponded to those in adults following a daily 50 and 100 mg dose, respectively. Thus, in pediatric patients, anidulafungin can be dosed based on body weight.<sup>175</sup>

### Micafungin

Micafungin is licensed only in the US for prevention of *Candida* infections in patients undergoing hematopoietic stem cell transplantation and for treatment of esophageal candidiasis;<sup>176</sup> regulatory approval for invasive candidiasis and a pediatric label are expected in the near future.

Micafungin has been studied in an open-label, sequential group, dose escalation study of empiric therapy in 70 febrile granulocytopenic children and adolescents aged 2–17 years. In this study, micafungin was well tolerated at dosages ranging from 0.5 to 3.0 mg/kg/day; pharmacokinetics were linear and pharmacokinetic parameters were similar to those observed in adults<sup>177</sup> (Table 22-9). A phase 1, single-dose, multicenter, open-label, sequential dose trial has investigated the safety and pharmacokinetics of micafungin (0.75 mg/kg, 1.5 mg/kg and 3.0 mg/kg) in 18 premature infants weighing >1000 g. Pharmacokinetics in preterm infants appeared to be linear. However, premature infants >1000 g on average displayed a shorter half-life and a more rapid rate of clearance compared with published data in older children and adults. All doses of micafungin were well tolerated and no serious drug-related adverse events were observed.<sup>178</sup>

A multinational, non-comparative study investigated the activity of micafungin alone ( $n = 2$ ) or in combination ( $n = 56$ ) with other agents in 58 pediatric patients with proven or probable invasive aspergillosis. Fifty-four patients had failed prior therapy. The mean daily dose was  $2.0 \pm 1.2$  mg/kg/day, and the mean duration of dosing was  $67 \pm 85$  days. Overall response was 26/58 (45%).<sup>179</sup> The efficacy and safety of micafungin were further investigated in the pediatric subpopulation of a large, randomized, double-blind, phase 3 trial in patients with invasive candidiasis or candidemia. Patients were randomized to receive intravenous micafungin (2 mg/kg/day) or liposomal amphotericin B (3 mg/kg/day) for a minimum of 14 days. In the intent-to-treat analysis, 36/52 patients randomized to receive micafungin (69.2%) and 40/54 (74.1%) randomized to receive liposomal amphotericin B were successfully treated. The incidence of serious adverse events (3.8% versus 9.3%) and the rate of patients discontinuing therapy because of an adverse event (3.8% versus 16.7%) were lower in micafungin-treated patients.<sup>180</sup>

Thus, a considerable number of pediatric patients have been included in clinical trials with micafungin without evidence for differences in safety and tolerance as compared to adults.

## Conclusion

Pediatric age groups display important differences in host biology, predisposing conditions, epidemiology and presentation of fungal infections relative to the adult population. Over the past decade, major advances have been made in the field of medical mycology. Most importantly, an array of new antifungal agents has entered the clinical arena. Although the final pediatric approval of several of these agents remains to be established, pediatric development is moving forward at a steady pace. Invasive fungal infections will remain important causes for morbidity and mortality in immunocompromised pediatric patients. The availability of alternative therapeutic options is an important advance. At the same time, however, antifungal therapy has become increasingly complex. In addition to information on prior antifungal therapies, microbiologic data, existing co-morbidities and co-medications, a detailed knowledge of the available antifungal armamentarium and contemporary clinical trials is needed more than ever in the management of the individual patient.

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# Oral fungal infections

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With the exception of candidiasis, which is generally a superficial infection of oral epithelial surfaces, fungal infections of the mouth, face or neck are unusual. They occasionally occur in isolation but more often represent local involvement of a more disseminated infection. This chapter will review the more common features of such infections. Fungal infection of the sinuses is addressed elsewhere.

## Candidiasis

*Candida* species are normal inhabitants of the human gastrointestinal tract and may be recovered from up to one-third of the mouths of normal individuals.<sup>1</sup> The most common species associated with mucosal infection of the mouth is *Candida albicans*, although in certain circumstances other species (*C. dubliniensis*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. guilliermondii*, *C. kefyr*, and *C. krusei*) are isolated.<sup>2</sup> Although *C. albicans* can be cultured from the mouths of non-infected normal individuals, it does not cause disease unless predisposing factors exist to allow infection to become established.

The determinants of the protective host immune response to *Candida* infection have not been entirely established; yet in terms of human susceptibility there is a clear dichotomy between susceptibility to local disease and to systemic invasive disease. Adequate neutrophil function seems to protect against invasive candidiasis. However, local factors and an intact T cell-mediated defense system seem more important for protection against mucosal candidiasis.<sup>3</sup> A rare congenital syndrome, chronic mucocutaneous candidiasis, which is characterized by recurrent skin and mucosal *Candida* infections, is associated with deficient T cell responses to *C. albicans*.<sup>4</sup> Although the level of immunosuppression may be the most important, other host factors have been associated with protection against *Candida* infections. These include blood group secretor status, salivary flow rates, epithelial barrier, antimicrobial constituents of saliva, presence of normal bacterial flora, and local immunity.<sup>5</sup> Thus derangement in these can lead to increased risk for *Candida* infections.

Saliva seems to be an important constituent of the protection against *Candida* infections; candidiasis is almost inevitable in patients with xerostomia.<sup>6</sup> It is unclear, however, whether it

is the reduction in salivary flow or loss of specific anti-*Candida* factors that accounts for the increased risk of infection. Nutritional deficiencies can also predispose to oral *Candida* infections.<sup>7</sup> The best documented is iron deficiency<sup>8</sup> which, when chronic, is associated with mucosal atrophy, which may be an important predisposition. In addition, iron deficiency may cause immune defects or affect the function of important local enzymes that are iron dependent.

Diabetes mellitus has been linked to an increased risk of *Candida* infections for a long time.<sup>9</sup> Although the precise mechanisms are unclear, the rate of *Candida* infections does not seem to be related to glycemic control.<sup>10</sup> Some studies have shown increased adherence of *Candida* species to the oral mucosa of diabetic patients, which may increase risk of colonization.<sup>10</sup> Other conditions known to predispose to mucosal candidiasis are listed in Table 23-1.

The association between an intact cell-mediated immunity and predisposition to oral *Candida* infection finds its clearest expression in AIDS. Oropharyngeal candidiasis (OPC) was among the initial manifestations recognized in association with human immunodeficiency virus (HIV) infection.<sup>11</sup> Furthermore, the occurrence of oropharyngeal candidiasis may be a sentinel event presenting months or years before more severe opportunistic disease and indicating progressive loss of immunity. Several studies suggest that additional impairments in a number of anti-*Candida* host defense mechanisms occur in persons with HIV infection, which may increase the risk of infection.<sup>12</sup>

The incidence of *Candida* infections in HIV-infected individuals without advanced immunodeficiency who are not on antiretroviral therapy has been reported as varying from 7% to 48%.<sup>13</sup> The incidence increases as the CD4+ lymphocyte count decreases, with up to 92% of patients demonstrating evidence of oropharyngeal candidiasis at some time if their immunodeficiency is untreated. In patients with CD4+ lymphocyte counts less than 100/mm<sup>3</sup>, more than 60% will develop oropharyngeal candidiasis each year. Evidence from studies in the early era of HIV suggests that from 30% to 80% of patients without antiretroviral therapy or antifungal prophylaxis experience at least one recurrence at some time.<sup>14</sup> Despite the frequency of mucosal disease, disseminated or invasive infections with *Candida* and related yeasts are not more common in this population.

**Table 23-1** Risk factors for the development of oropharyngeal candidiasis

Immunosuppression
HIV infection
Chronic mucocutaneous candidiasis
Neutropenia
Drugs
Cytotoxic chemotherapy
Corticosteroids
Broad-spectrum antimicrobial agents
Anticholinergics
Diabetes mellitus
Nutritional deficiencies
Iron deficiency
Malnutrition
Prior or current local pathology
Dentures
Xerostomia
Infancy

In more recent years, the prevalence of oral *Candida* infection in HIV-infected patients has declined. Two factors have contributed to this. The first is the widespread use of antifungal agents, particularly the azole antifungals.<sup>15</sup> More importantly, the introduction of potent antiretroviral therapy has resulted in a significant decline in the incidence of a number of opportunistic illnesses (e.g., *Pneumocystis jiroveci* pneumonia and cytomegalovirus) and the mortality of AIDS.<sup>16</sup> Not unexpectedly, the historically high incidence of mucocutaneous candidiasis has also declined as more patients are treated with more potent antiretroviral therapy.<sup>17-19</sup>

Oropharyngeal candidiasis is also a particular problem in patients undergoing cancer chemotherapy.<sup>20</sup> Antineoplastic drugs can affect the number of circulating neutrophils but also interfere with lymphocyte and monocyte function. Radiotherapy may cause xerostomia. Many patients undergoing chemotherapy receive prophylactic or therapeutic broad-spectrum antibacterials, which also disrupt the normal host defenses and predispose to *Candida* infection.

Medications other than chemotherapy or antibacterials can predispose to the development of candidiasis. Corticosteroids are immunosuppressive and candidiasis can occur even with the metered-dose preparations used to treat asthma and allergic rhinitis.<sup>21</sup> Anticholinergic agents, such as tricyclic antidepressants, decrease salivary flow and thus also predispose to *Candida* infection. Other diseases in which decreased salivary flow is a feature (such as Sjogren's syndrome) are also associated with a predisposition to candidiasis.

Denture stomatitis has been well recognized as a complication of wearing dentures.<sup>22</sup> It is a chronic inflammatory condition caused by the trauma of ill-fitting dentures or possibly by a allergic response to denture material with a superimposed *Candida* infection. It is important to treat the denture and the patient, because it can serve as a nidus for *Candida* growth.

## Microbiology

As noted earlier, *C. albicans* causes most infections. Most disease is caused by organisms that are part of the normal flora of an individual, although rare cases of person-to-person transmission have been documented.<sup>23</sup> Recurrent disease can result from the same species or strains of *Candida* or because of a change in either.<sup>24,25</sup> In patients with HIV infection, *C. dubliniensis* has been increasingly recognized as an important cause of oropharyngeal disease.<sup>26</sup> This species is phenotypically very similar to *C. albicans* and specialized techniques are required for its definitive identification. The emergence of different strains or species is more likely in persons exposed to prolonged or multiple courses of suppressive antifungal therapy and has generally been described in association with advanced HIV disease.<sup>27</sup>

## Clinical features

Although usually associated with slight morbidity, oropharyngeal candidiasis can be clinically significant. Severe oropharyngeal candidiasis can interfere with the administration of medications and adequate nutritional intake, and may spread to the esophagus.<sup>28</sup> Symptoms may include burning pain, altered taste sensation, and difficulty swallowing liquids and solids. Many patients are asymptomatic. Most cases of oropharyngeal candidiasis are diagnosed on the basis of their clinical appearance. Lesions are most likely to be seen on the dorsum and sides of the tongue or on the buccal, palatal, gingival, and pharyngeal mucosa. In HIV-infected patients, in contrast to most other patients, candidiasis is often present in multiple oral sites. Indeed, this is the typical presentation of *Candida* infection in patients with AIDS, who generally have multiple oral foci. Three types of presentation of oropharyngeal candidiasis within the mouth are recognized, as follows.

1. Pseudomembranous candidiasis or thrush, which appears as painless white creamy plaques on the mucosa with underlying erythema. When plaques are wiped off, the underlying mucosa is red and erythematous and may bleed slightly. Any part of the oral mucosa may be involved.
2. Atrophic (erythematous) candidiasis, which appears as red patches most commonly on the palate or as an atrophic depapillated tongue. This form of *Candida* infection is particularly associated with xerostomia, nutritional deficiencies, and local trauma and is well recognized in patients with dentures.
3. Chronic hyperplastic candidiasis (leukoplakia) involving the tongue, inner commissures of the lips or buccal mucosa. Unlike pseudomembranous infections, these lesions cannot be wiped off. Two factors that have been particularly associated with this form of *Candida* infection are smoking and blood group secretor status (non-secretors are more prone to infection). In the more chronically infected patients, food or tobacco may stain the lesions brown. In the HIV-infected patient, this form of candidiasis must be distinguished from oral hairy leukoplakia, which is caused by Epstein-Barr virus infection.

*Candida* can also involve the commissures of the lips with red fissured lesions that crack and crust and are associated with discomfort, burning or pain (angular cheilitis or perleche).

The major complication of oropharyngeal candidiasis is spread to the esophagus. The diagnosis of *Candida* esophagitis can be made empirically in patients with oropharyngeal disease who have symptoms suggestive of esophageal involvement, i.e., dysphagia, odynophagia, and retrosternal pain.<sup>29</sup> In such situations, invasive procedures such as endoscopy can be reserved for patients who fail to respond to empiric systemic antifungal therapy.

Recovery of an organism is not required to make the diagnosis of candidiasis. Oropharyngeal cultures often demonstrate *Candida* species but alone are not diagnostic, because colonization is common. Scrapings of active lesions, examined with 10% potassium hydroxide, demonstrate characteristic pseudohyphae and budding yeast. The appearance of the lesion and the presence of yeast forms on microscopic examination are enough to confirm the diagnosis. Culture is usually not necessary unless the lesions fail to clear with appropriate antifungal therapy. Many microbiology laboratories report yeast cultures as either *C. albicans* or non-*albicans* species based on the germ tube test, and the clinician must request further characterization if desired. Biopsies are rarely helpful or indicated. Clinicians often make a presumptive diagnosis of oropharyngeal candidiasis by documenting clearance of typical lesions with antifungal therapy.

## Treatment

There is a wide variety of agents that are effective for the treatment of candidiasis (Table 23-2). Treatment of oropharyngeal candidiasis is relatively simple, with most types responding well to therapy. In trials, the response rate varies from 34% to 95%. However, studies of antifungal treatment for mucocutaneous candidiasis suffer from one or more weaknesses, such as small numbers of patients, heterogeneous populations, short follow-up, and a non-blinded design. In the particular case of HIV-associated *Candida* infection, studies have not stratified patients by CD4+ lymphocyte count. This is important, because persons with low CD4+ lymphocyte counts seem to respond more slowly to treatment, and have lower rates of fungal eradication and higher relapse rates than persons with less advanced disease.

Overall, there are few clinical differences in randomized studies comparing topical treatments with oral systemic therapy or comparing different oral systemic therapies;<sup>30</sup> the one exception may be nystatin suspensions, which have been shown to be inferior to other topical or systemic treatment, possibly because of compliance issues.<sup>31</sup> Thus, it should be anticipated that at least 80% of patients with uncomplicated disease will respond to treatment and it is reasonable to conclude that clotrimazole troches, ketoconazole, fluconazole, and itraconazole are probably equivalent in the acute treatment of most cases of oropharyngeal candidiasis. Moderate or severe episodes, however, typically require systemic therapy, and esophagitis always requires systemic therapy.

The duration of therapy is also variable. In uncomplicated infection there has been a tendency to try and shorten the course of therapy. It does seem that, in general, courses of the systemic azoles (fluconazole and itraconazole) can be shorter than courses of the topical treatments. However, it is extremely difficult to make predictions for a given patient, because host factors (such as degree of immunodeficiency, local conditions

**Table 23-2** Therapeutic options for mucosal candidiasis

Medication	Dosage	Important toxicities
Clotrimazole troches	10 mg 4–5/d × 7–14 d	Altered taste, GI upset
Nystatin suspension	100,000 units/ml 5 ml qid × 7–14 d	GI upset
Amphotericin B suspension	1–2 ml qid × 7–14 d	Altered taste
Ketoconazole	200 mg/d × 7–14 d	GI upset, hepatitis, endocrine effects
Itraconazole	100 mg/d × 7–14 d	GI upset, hepatitis
Itraconazole suspension	10 ml/d × 7–14 d	GI upset, hepatitis
Fluconazole	100 mg/d × 7–14 d	GI upset, hepatitis
Fluconazole suspension	10 ml/d × 7–14 d	GI upset, hepatitis

in the mouth) may be critical in determining the response to treatment. In general, patients should receive itraconazole or fluconazole for at least 7 days, and the topical agents should probably be given for 14 days. Shorter courses are effective in many patients and it is not unusual for patients to have symptomatic improvement within 1 or 2 days of starting therapy.

There are a few trials of prophylactic antifungal therapy for mucocutaneous candidiasis in persons with HIV infection.<sup>30</sup> Most have been relatively small and of short duration but have demonstrated that fluconazole is effective in preventing recurrent disease. In a large trial of fungal prophylaxis, daily fluconazole was more effective than clotrimazole in preventing mucosal candidiasis and invasive infections such as cryptococcosis; however, in the median 3 years of follow-up, more than 10% of patients receiving fluconazole developed *Candida* infection.<sup>32</sup> Thus most studies indicate that fluconazole in variable doses has activity in the prevention of recurrent disease. However, chronic azole therapy fails to eradicate *Candida* colonization and recurrent infection still occurs, especially with progressive immunodeficiency.

At this point, most experts do not recommend universal antifungal prophylaxis<sup>33</sup> and its necessity has diminished greatly in the era of potent antiretroviral therapy.<sup>15</sup> The most effective method of prevention of mucocutaneous candidiasis is the reversal of the immunodeficiency with HIV infection.<sup>17</sup> The use of secondary prophylaxis should be individualized. Some experts do recommend prophylaxis in those who have had esophageal candidiasis<sup>33</sup> but long-term prophylaxis is not needed if the patient receives effective antiretroviral treatment. Other possible interventions include smoking cessation, good oral hygiene, and avoidance of unnecessary antibiotics and steroids.

The development of candidiasis should be taken as an indication to initiate or change antiretroviral therapy.



Candidiasis is a sign of the failure of the immune system, which is usually well controlled with effective antiretroviral therapy. The development of *Candida* infection in a patient already taking antiretroviral therapy suggests non-adherence or the development of resistant HIV or both. HIV-positive patients with occasional disease or infrequent recurrences of oropharyngeal candidiasis (<3 episodes per year) can be treated for each episode. An alternative approach is to provide the patient with a supply of antifungal medications that can be initiated at the earliest sign of recurrence. Those with frequent recurrences or complications that result in nutritional impairment or severe esophageal disease may be a group that will benefit from secondary prophylaxis, particularly as the CD4+ lymphocyte count declines. A recent randomized clinical trial showed that, in HIV-infected patients with access to active antiretroviral therapy, continuous fluconazole therapy was more effective than episodic fluconazole therapy and was not associated with significant risk of fluconazole-refractory *Candida* infection.<sup>15</sup>

In summary, continuous use of antifungal agents should be reserved for those persons with frequent or severe recurrences of mucosal candidiasis to avoid the emergence of drug resistance, avoid drug interactions, simplify already complex drug regimens, avoid drug toxicity, and lower the cost of treatment.<sup>33</sup>

Measures to decrease the frequency of *Candida* infections in patients with neutropenia have included local therapy designed to decrease *Candida* colonization and systemic chemoprophylaxis. In general, oral regimens that are designed to reduce the amount of *Candida* in the gastrointestinal system (using polyenes such as nystatin or amphotericin B, or azoles, e.g., clotrimazole) have had moderate activity in the prevention of oropharyngeal infection and little or no effect on systemic candidiasis.<sup>34</sup> Compliance is a major problem with these regimens. Fluconazole at dosages of 50–400 mg/day has been effective in preventing oropharyngeal candidiasis and decreasing colonization with *Candida*. At the higher doses, fluconazole has been shown to decrease systemic candidiasis in adult patients undergoing bone marrow transplantation and in reducing the need for systemic amphotericin B. The situation is less clear in other neutropenic patients. Because fluconazole is less active against *C. glabrata* and *C. krusei*, increased colonization and, at some centers, increased infection with these species has been reported<sup>35</sup> when fluconazole is used routinely for prophylaxis.

### Azole-resistant candidiasis

One of the consequences of continuous suppressive antifungal therapy in HIV-infected patients has been the emergence of resistant disease.<sup>36</sup> Resistance has been described to all azoles and tends to occur in persons with advanced HIV disease (CD4+ lymphocyte counts <50/mm<sup>3</sup>), who have been exposed to antifungal therapy on a chronic basis.

Several mechanisms of resistance to azole antifungals have been described, including target alteration, reduced cell permeability, and active efflux of the drug out of the cell. Some yeasts seem to be resistant to only one drug, whereas others are multidrug resistant. Azole resistance has been demonstrated in yeasts that contain alterations in the enzymes that were the target of their action or were involved in ergosterol biosynthesis.

The cytochrome P450-dependent 14 $\alpha$ -sterol demethylase (P450<sub>DM</sub>) and the  $\alpha$ 5,6 sterol desaturase are two enzymes that when altered result in azole resistance. Reduced cell permeability is another mechanism of azole resistance. Finally, active efflux of drug has also been observed and may be fluconazole specific or involve mechanisms that lead to efflux of all azole antifungals.

In 60–75% of cases of resistant candidiasis, *C. albicans* can be cultured from the mouth. Patients on fluconazole suppressive therapy are more likely to have infection caused by other species such as *C. glabrata*, *C. parapsilosis*, and *C. krusei*. These organisms tend to be less susceptible to fluconazole. However, susceptibility testing is rarely used clinically for management of OPC, because treatment decisions are usually based on clinical grounds. However, testing of isolates from patients with oropharyngeal candidiasis refractory to standard doses of fluconazole can be used to differentiate true resistance from other causes of failure.

Infection with fluconazole-resistant candidiasis is often difficult to manage and may cause significant morbidity. The clinical expression of disease is identical to that seen in patients with sensitive infection but it tends to be progressive and more symptomatic because of the lack of effective therapy. Thus, esophagitis seems to be very common. There is no information to suggest that resistant infection is more virulent.

The first thing that needs to be done in managing a patient with clinical failure to apparently adequate doses of fluconazole is to verify that the drug is indeed being taken as prescribed. Is the patient compliant? Are there adequate serum levels? Because this condition occurs almost exclusively in patients with HIV disease, antiretroviral therapy should be optimized. There are many anecdotal reports of refractory candidiasis responding solely to the introduction of more effective antiretroviral therapy, and indeed, the frequency of this problem has diminished dramatically with the widespread use of more potent treatment for HIV.

If new antifungal therapy is needed, other azoles may be efficacious because some fluconazole-resistant isolates retain sensitivity to itraconazole, voriconazole, and posaconazole. The cyclodextrin formulation of oral itraconazole has been shown to be effective in a small number of patients, with response rates of 60–70%.<sup>37</sup> Posaconazole is also effective in this situation<sup>38</sup> with 75% response rates using either oral posaconazole (400 mg twice daily) for 3 days followed by oral posaconazole (400 mg once daily) for 25 days or oral posaconazole (400 mg twice daily) for 28 days. The oral suspension of amphotericin B, given in relatively high doses (5 ml qid), is also effective in azole-resistant oropharyngeal candidiasis.<sup>39</sup> The echinocandins are probably the treatment of choice for more severe refractory candidiasis, especially if the esophagus is involved. Caspofungin (70 mg loading dose followed by 50 mg daily, all given IV) has been shown to have response rates of 70–80% for esophageal candidiasis,<sup>40,41</sup> including that associated with fluconazole resistance.<sup>42</sup> Similarly, anidulafungin (100 mg load then 50 mg/day<sup>43</sup>) and micafungin (150 mg/day<sup>44</sup>) are also active in oropharyngeal and esophageal candidiasis. Finally, IV amphotericin B can be used in severe disease, especially esophagitis, for patients who fail other treatments. A short course of therapy at low doses (0.3 mg/kg for 7–14 days) is usually effective, although relapses are common without some form of maintenance therapy.

## Aspergillosis

*Aspergillus* infection is prevalent in immunocompromised hosts. The organisms are found commonly in the environment and the conidia probably are commonly inhaled. However, unless the patient has some preexisting risk factors, invasive infection is rare. The respiratory tract is the most common primary site of this infection, and invasive aspergillosis involving the head and neck is well recognized. Among the orofacial manifestations are invasive sinusitis in the immunocompromised host, chronic indolent sinusitis, aspergilloma in the maxillary antrum, and oral lesions.

*Aspergillus* infection of the upper respiratory tract can involve the gingiva, hard palate, paranasal sinuses, and nasal mucosa. Primary oral aspergillosis typically involves the marginal gingiva or palate. The patient, usually granulocytopenic, complains of severe gingival pain with associated fever. The infection begins as violaceous lesions in the marginal gingiva in the absence of surrounding edema. Early-stage lesions are usually solitary. Within a few days, the lesion progress to necrotic ulcers covered by pseudomembranes. The infection rapidly spreads to involve alveolar bone and facial muscles. In some patients with prolonged granulocytopenia, a noma-like lesion may characterize the infection. The edentulous alveolar ridge has a lower frequency of *Aspergillus* infection, suggesting that periodontal disease may contribute to the development of oral aspergillosis.

Sinus infection is common with *Aspergillus* infection. The mildest form is a non-invasive fungus ball causing chronic obstruction of the maxillary antral sinus. Less commonly, a more chronic indolent invasive sinusitis develops. Typically such patients have chronic sinusitis unresponsive to antibacterial therapy. Acute invasive sinusitis is seen primarily in immunocompromised hosts, such as those with severe neutropenia or AIDS. Patients usually have the classic features of sinusitis (fever, facial pain and swelling, nasal discharge, and headache). As in other sites, *Aspergillus* infection tends to invade locally. Computed tomography (CT) of the sinuses will usually show bony erosion, and penetration into adjacent tissues such as the brain or the orbit can occur. Bony extension inferiorly may involve the hard palate, leading to oroantral openings.

Early diagnosis is important in obtaining optimal therapeutic results in the immunocompromised host. Delayed diagnosis and treatment may lead to progression of the disease and an ultimately fatal outcome. Presentation of this infection may vary, so definitive diagnostic procedures such as biopsy should be performed. Co-infection with bacteria, viruses, and other fungi can make early diagnosis more difficult. In addition, non-infectious complications associated with transplantation, such as graft-versus-host disease, may further complicate the picture. A combination of microbiologic and histologic examination of the affected tissue is necessary to make the diagnosis. Pathologic examination of suspicious lesions is necessary to confirm the diagnosis and should be done early. Diagnosis is made by biopsy, with staining with Gomori methenamine silver or periodic acid-Schiff stain showing narrow, branching septate hyphae. Although cultures may be positive, in view of the ubiquitous nature of the fungus, isolation of the fungus is not proof of invasive disease. However, in neutropenic patients or others at high risk of disease, a positive culture often

indicates invasion and may be sufficient to warrant initiation of therapy. Serologic testing is unhelpful in the diagnosis of invasive aspergillosis.

Radiologic studies (e.g., CT of sinuses) help to define the extent of tissue invasion of the infection. Sclerotic bony changes reflect the relative chronicity of the infection. Occasionally, culture from a patient with chronic sinusitis may grow *Aspergillus* species. In the absence of bony invasion on biopsy or CT scan, culture may represent colonization, and aggressive therapy may not be required.

Management of head and neck aspergillosis involves a combination of surgical debridement of the involved tissue and aggressive antifungal therapy.<sup>45</sup> Given the availability of several agents with activity against aspergillosis (amphotericin B, voriconazole and posaconazole, and the echinocandins<sup>46-48</sup>), expert advice as to optimal antifungal therapy should be sought.

## Zygomycosis

Fungi of the family Mucoraceae of the class Zygomycetes are ubiquitous and are associated with soil and decaying matter. These organisms can be cultured readily from these environmental sources but are also found commonly in cultures from the nose or mouth in patients without disease. Infection with these organisms is almost always a consequence of some form of immunocompromise such as neutropenia accompanying leukemia or chemotherapy, diabetic ketoacidosis, burns, immunosuppressive therapy, especially corticosteroids, and the acquired immunodeficiency syndrome.<sup>49</sup>

The oral cavity may be involved as a progression of rhinocerebral zygomycosis, which is the form classically associated with diabetic ketoacidosis. This usually commences in the nasal cavity or paranasal sinuses. Patients initially are seen with pain and nasal discharge. Local invasion is typical; invasion of the palate produces a black necrotic oral ulcer on the roof of the mouth; invasion of the orbit leads to orbital cellulitis, proptosis, and impaired ocular movement. The most devastating complication is intracranial invasion, either through the cribriform plate or by penetration of the ophthalmic vessels.

Diagnosis is confirmed by histologic demonstration of tissue invasion by the typical broad irregular non-septate to sparsely septate branching hyphae that classically invade blood vessels with consequent thrombosis and tissue infarction. The organisms are best seen with methenamine silver or periodic acid-Schiff staining. The prognosis of rhinocerebral mucormycosis is poor; both surgical debridement and systemic antifungal therapy are required.<sup>49</sup> Amphotericin B has been the mainstay of antifungal treatment but posaconazole shows very promising activity in zygomycosis.<sup>50</sup>

## Cryptococcosis

*Cryptococcus neoformans* is a ubiquitous yeast found in soil contaminated with avian excreta. The central nervous system is the most common site of disseminated cryptococcal infection, usually seen as meningitis. Although disseminated disease is well recognized, involvement of the mouth is unusual. Oral involvement has been reported as the presenting feature of the

infection in a number of patients, especially associated with HIV infection. Lesions may be found on the tongue, gingiva, hard and soft palate, pharynx, buccal mucosa, and tonsils; they are typically seen as ulcers or nodules of granulation tissue. Lesions on the buccal mucosa may have a thrush-like appearance. Ulcerating lesions may be found on the lateral border of the tongue with rolled, elevated borders with minimal inflammation present and marked induration beyond the border of the ulcer.

Diagnosis is confirmed by isolation of *Cryptococcus* from a sterile body site, by histopathology or by detection of cryptococcal capsular antigen. Histopathology showing encapsulated yeast staining with mucicarmine or Gomori methenamine silver is diagnostic of *Cryptococcus*. Two histologic patterns may be seen: proliferating yeast with minimal tissue reaction and no necrosis, and granulomatous pattern without caseation. The cryptococcal antigen test has a greater than 95% specificity and sensitivity. Serum antigen detection may be useful in the initial diagnosis of disseminated infection such as occurs with oral involvement, but the utility of serial antigen determinations during management is less clear.

Disseminated cryptococcal infection should be managed in the same way as cryptococcal meningitis (see Chapter 9).

## Histoplasmosis

*Histoplasma capsulatum* is a dimorphic fungus that grows as a mycelial form at room temperature and as a yeast at 37°C. Infection is acquired by inhalation of conidia present in the environment. The organism is endemic in North America, in particular the Mississippi and Ohio River valleys, and in Central and South America. Occasional cases of endemic histoplasmosis occur in Africa, Australia, India, and Eastern Asia. *H. capsulatum* var. *duboisii* is found in equatorial Africa and particularly associated with skin lesions.

Although most cases of histoplasmosis are acute and self-limited, a more chronic disseminated form occurs, especially in immunocompromised patients, and can spread hematogenously to affect multiple sites, including the oropharynx.<sup>51,52</sup> The most common site of involvement is the tongue, followed by the buccal mucosa and then the larynx. The tongue and buccal mucosa are involved in 40–75% of adults and in 18% of children with disseminated disease. Lesions may be found in multiple sites in the same person. Lesions of the tongue and buccal mucosa are characterized by firm, painful ulcers with heaped-up edges. Proliferative lesions with verrucous or plaque-like appearance may be seen in the early stages, with central ulceration occurring if the lesions remain untreated. Ulcers in children may be shallow and mimic aphthous ulcers. Involvement of the oropharynx may be the only sign of disseminated infection. Sore throat, painful mastication, hoarseness, gingival irritation, and dysphagia are common presenting features. These may be associated with weight loss in the absence of other constitutional symptoms. These chronically enlarging painful ulcerations need to be differentiated from carcinoma or other chronic infections such as tuberculosis. Oral manifestations of histoplasmosis are reported to be the initial manifestation of AIDS in HIV-infected patients. Occasionally isolated lesions have been recorded in otherwise healthy persons without obvious systemic histoplasmosis.

The diagnosis can be made by taking a swab from the center of the lesion for microscopy and culture. Special stains using Gomori methenamine silver, Giemsa or periodic acid-Schiff usually demonstrate macrophages containing yeast forms. Biopsy of the lesion shows granulomata with central necrosis, which may be difficult to distinguish from tuberculous granuloma; however, special stains of the tissue can identify the yeast. Urine *Histoplasma* antigen may be positive and diagnostic in patients with disseminated disease.<sup>53</sup> Complement fixation tests with titers >1:32 are also diagnostic.<sup>52</sup>

Oral histoplasmosis is a sign of disseminated infection, and amphotericin B 0.5–1 mg/kg for 2 weeks followed by itraconazole is considered the treatment of choice for patients with severe disease.<sup>52,54,55</sup> Itraconazole is also effective as primary therapy for progressive disseminated histoplasmosis, 300 mg bid for 3 days followed by 200 mg bid indefinitely. Patients should be treated for a minimum of 3–6 months. To prevent relapse in HIV-infected patients, suppressive therapy is recommended. Itraconazole 200 mg bid is recommended and is associated with a relapse rate as low as 5% at a median follow-up of 2 years.<sup>56</sup> Suppressive therapy can be discontinued if patients have recovery of immune function after effective antiretroviral therapy.<sup>57</sup>

## Blastomycosis

Blastomycosis is an endemic fungal infection caused by *Blastomyces dermatitidis*. It may present clinically as a self-limited primary pulmonary infection, chronic pulmonary infection or disseminated disease. Despite the similarities with histoplasmosis, oropharyngeal and laryngeal involvement is much less common with blastomycosis. Isolated mucosal involvement of the hard palate, gingiva, and tongue may be seen and is usually associated with pulmonary infection. The patient presents with oropharyngeal pain. The appearance of the lesions may vary. Advanced lesions are characterized by a proliferative verrucous growth with scarring. In contrast to histoplasmosis, ulceration is uncommon. The patient may experience weight loss, low-grade fever, and respiratory symptoms, reflecting the disseminated nature of the presentation.

Biopsy of the oral lesions should be performed and stained with periodic acid-Schiff or Gomori methenamine silver. Diagnosis can be made by demonstrating typical yeast forms with single broad-based buds. Because oral involvement usually indicates disseminated disease, amphotericin B is the drug of choice.<sup>58</sup> A total dose of 1 g over 2–4 weeks results in cure in 90% of patients. Patients need to be followed carefully for months after treatment to ensure that relapse does not occur. Relapse of blastomycosis after therapy occurs rarely and seems to be dose dependent. Itraconazole is an effective alternative and should be given for 4–6 months.<sup>54,59</sup>

## Coccidioidomycosis

Coccidioidomycosis is a systemic fungal infection endemic in Southwestern United States, Mexico, Central and South America. The causal agents are *Coccidioides immitis* (predominantly found in California) and *C. posadasii* (endemic elsewhere), dimorphic fungi that exist in the soil in the mycelial phase.



Maturation of the fungus results in formation of hyphae and arthroconidia, which are easily aerosolized and inhaled. In most cases, this produces a relatively mild self-limited illness, generally involving the lungs. Disseminated disease is rare. Pregnant women, African-Americans, Filipinos, and Hispanics are more prone to disseminated disease, as are immunocompromised patients, such as those with HIV infection.<sup>60</sup> Dissemination typically involves the skin, bone, and meninges. Oral involvement is rare.

Diagnosis is made by culture of the organism from clinical specimens or by demonstration of the typical spherule on histopathologic examination. The spherule can be identified with stains such as Gomori methenamine silver or Papanicolaou stain. Coccidioidal serologic tests may be positive but as many as one-quarter of patients with disseminated disease have negative serologic tests. Amphotericin B remains the treatment of choice for disseminated infection, although both itraconazole and fluconazole are active and may be used for chronic management.

## Other fungal infections

**Paracoccidioidomycosis**, or South American blastomycosis, is endemic in parts of Colombia, Venezuela, Brazil, Argentina, and Uruguay. The causative organism is the dimorphic fungus *Paracoccidioides brasiliensis*. Paracoccidioidomycosis is increasingly seen in patients with AIDS in the endemic area.<sup>61</sup> Like the other endemic mycoses, the primary pathology is usually pulmonary and often self-limited. In more chronic disease, patients have cough, hemoptysis, and dyspnea. Granulomatous oral ulcers are common with the more chronic form of infection.<sup>62</sup> Diagnosis is made by biopsy of the lesion. Treatment is with amphotericin B, itraconazole or sulfonamides.

**Sporotrichosis** due to *Sporothrix schenckii* usually is the result of local inoculation. Disseminated sporotrichosis has been reported rarely. The usual presentation is one of diffuse cutaneous disease with polyarthritis, but the mouth occasionally may be involved.<sup>59</sup> Amphotericin B is the treatment of choice. Itraconazole may also be effective.

**Geotrichosis** is an infection of the bronchi, lungs, and mucosa caused by a yeastlike fungus, *Geotrichum candidum*. In the mouth it can produce thrush-like lesions.<sup>63</sup>

**Penicilliosis** due to disseminated infection with the mould *Penicillium marneffei* has been reported increasingly in patients with AIDS from Southeast Asia, especially northern Thailand and southern China.<sup>64</sup> Skin lesions are an extremely common manifestation of this infection, and oral involvement occasionally can be seen as part of disseminated disease.

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## Cutaneous and subcutaneous mycoses

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The cutaneous mycoses can be divided in two groups: the superficial mycoses, which are fungal infections that affect the skin only, and subcutaneous mycoses which involve the tissue beyond the skin.

The human skin is affected by multiple fungal organisms which can be classified according to the site of origin as zoophilic (fungi found primarily in animals which can be acquired by humans and develop inflammatory reactions), anthropophilic (found mainly in humans), and geophilic (organisms usually recovered from the soil that occasionally infect humans and animals; the inflammatory reaction is marked, limiting the spread in some cases and leaving scars).

There are multiple factors that affect the incidence of fungal infections within a particular population, including the atmospheric and geographic conditions, the immunocompetence of the host, the pathogenicity of the infectious agent, the level of education of the population at risk,<sup>1</sup> the activities of the host and the availability of medical treatment.<sup>2</sup> The development of clinical signs of fungal infections is related to the immune status of the host. One of the best examples of this correlation is the infections caused by *Candida* spp. which are much more frequent in immunocompromised patients. Fungal infections also account for an important number of office visits and treatment-related expenses.<sup>3</sup> Failure of appropriate treatment influences the progression of disease and development of resistance.

### Superficial fungal infections

The superficial cutaneous mycoses are common around the world. The usual causative organisms include the dermatophytes, yeasts, and non-dermatophyte moulds. Although the majority of mycotic infections affecting the skin are caused by the dermatophytes, there seems to be an emergence of new species and pathogens involved in skin disease which pose important diagnostic and therapeutic challenges to the physician.<sup>4</sup>

It is important to note that the relationship between the host (particularly skin tissue) and the fungal organisms is complex and changes constantly due to constant competition for nutrients between different species in a difficult environmental niche such as the skin. The organisms that lack the ability to

adapt to these environmental changes are usually displaced by more evolved organisms.<sup>5</sup>

### Dermatophytosis

The superficial fungal infections are confined to the stratum corneum and the appendages of the skin. The dermatophytes infect the keratinized structures of the skin (including the nails and hair shafts), using nutrients provided by keratin, even though they are not part of the human skin flora.<sup>6</sup>

The dermatophytoses are classified according to the part of the body involved and are usually designated “tinea” followed by the affected part. For example, tinea facie corresponds to dermatophytosis on the face, and tinea pedis is dermatophytosis of the foot. Usually the lesions caused by dermatophytes are called “ringworm” due to the anatomic shape.<sup>6</sup>

One of the most important factors that influence the presence of dermatophytes on the skin is the local conditions. A warm, moist and humid environment (such as the one found in occlusions) has been reported as an important factor predisposing to dermatophyte infections. Other risk factors include the socioeconomic status and personal hygiene of the patient.<sup>7</sup> Recent studies have also shown that providing healthcare education and professional foot care decreases the influence of these risk factors for developing dermatophyte infections.<sup>8-10</sup>

### Diagnosis

The diagnosis is suspected by the clinical presentation and it is confirmed by microbiologic techniques including light microscopic examination (using potassium hydroxide KOH) and/or culture.<sup>11</sup> The collection of the specimen for light microscopy can be done by scraping the affected area with a surgical blade or a sterile toothbrush.<sup>12</sup> The specimens for culture must be obtained by scraping and plucking some affected hair and inoculating them in the culture medium. The results from the cultures may take up to 4 weeks; and the rate of false negatives is high (up to 50%).<sup>11,12</sup> In cases of ectothrix infection (*Microsporum canis* or *Trichophyton schoenleinii*) the Wood’s ultraviolet light is useful (the infection can be detected by the presence of fluorescence).<sup>13</sup> Skin biopsy is rarely done, but it is helpful in cases of scarring alopecia of unknown origin. In these cases, special stains such as periodic acid-Schiff and





**Figure 24-1** Tinea capitis (courtesy of Adrina Motta MD, El Bosque University, Bogota, Colombia).



**Figure 24-2** Inflammatory tinea capitis (courtesy of Milton Gonzalez MD, Clinica EL Country, Bogota, Colombia).

methenamine silver may be needed to detect the presence of the infecting organism.<sup>14</sup>

## Tinea capitis

Tinea capitis is a superficial fungal infection more frequent in childhood and usually it affects children 3–7 years of age.<sup>6,12</sup> The estimated prevalence in the United States ranges from 3% to 8%<sup>12</sup> but some studies mention an increase in those figures.<sup>15</sup>

The organisms causing tinea capitis vary according to the region. In North America and the United States, *Trichophyton tonsurans* is the predominant organism (88.1%<sup>16</sup> for the USA and 76% for Canada).<sup>17</sup> In The Netherlands, the causative species include *T. verrucosum* (25.3%), *T. schonleinii* (24%), *T. violaceum* (17.3%), and *Microsporum canis* (14.4%). *T. rubrum* and *T. mentagrophytes* were also identified but in a minor proportion. *T. tonsurans* was not isolated in this study.<sup>18</sup> In Germany and Central Europe, *M. canis* is still the more prevalent agent.<sup>19,20</sup>

Tinea capitis is acquired by contact with other infected persons (at home or school), animals (dogs or cats), and inanimate objects (combs, caps, bedding, clothing and soil).<sup>21</sup> The incubation period is usually a week but sometimes is only a few days.<sup>22</sup> Nonetheless, the fungal organisms are viable for months.<sup>12</sup>

The hair from the scalp can be infected or invaded in three modes: ectothrix, in which the fungus is present around the exterior of the hair shaft, producing fluorescence on exposure to Wood's light, endothrix, in which the fungus is located within the hair shaft (Wood's light negative), and favus, in which the fungal organism is interspersed with air bubbles in the hair shaft.<sup>23</sup> Occasionally, the scalp can be infected and subcutaneous invasion occurs (a phenomenon designated kerion<sup>24</sup>).

### Clinical features

The lesions can be asymptomatic or produce some pruritus. The lesions start as a single plaque that grows in a centrifugal pattern. Depending on the grade of inflammation, the clinical features may vary. The usual presentation is the

non-inflammatory scalp ringworm (Fig. 24-1). This ringworm can be classified as: “gray” type, with one or more circular patches with marked scaling, “diffuse” scale type, characterized by widespread scaling similar to seborrheic dermatitis with minimal hair loss, and “black dot” type, characterized by areas of alopecia with hair shafts broken off at the surface of the skin without signs of inflammation.<sup>6,25</sup> Favus usually presents as scaly and erythematous patches with scutula (honeycomb-yellow cup). Occasionally, it becomes confluent and forms a hyperkeratotic mass which produces a cheese-like odor.<sup>25</sup>

The inflammatory types (Fig. 24-2) include:

- kerion, which consists of indurated and swollen plaques with vesicles and pustules localized in one spot. The lesions usually present a purulent discharge from the scalp sinuses as well as cervical lymphadenopathy and fever<sup>25</sup>
- “diffuse pustular” type which is characterized by multiple mycotic pustules on the scalp. It is accompanied by painful cervical lymphadenopathy.<sup>25</sup>

### Differential diagnosis

For non-inflammatory tinea capitis the differential diagnoses include seborrheic dermatitis, psoriasis, eczema, tinea amiantacea, alopecia areata, tricotillomania and traction alopecia.<sup>25</sup> The differential diagnoses of inflammatory tinea capitis include impetigo, neoplasia, abscess, bacterial folliculitis, perifolliculitis capitis abscedens et suffodiens and tinea amiantacea.<sup>25</sup>

### Treatment

Griseofulvin is considered the first-line therapy for tinea capitis. Some studies have compared fluconazole with griseofulvin and shown that the efficacy of fluconazole is no better than that of standard-dose griseofulvin. Nonetheless, fluconazole could be useful in patients with a contraindication or intolerance to high-dose griseofulvin.<sup>26</sup> A double-blind randomized trial comparing standard and double doses of terbinafine given in a pulsed protocol (1 week on, 3 weeks off) for the treatment of tinea capitis caused by *Microsporum* spp. proved to be effective, although an additional pulse treatment may be needed if clinical improvement is not evident at 8 weeks after initiating therapy.<sup>27,28</sup>



**Figure 24-3** Tinea corporis (courtesy of Adrina Motta MD, El Bosque University, Bogota, Colombia).

## Tinea corporis

Tinea corporis refers to all the dermatophyte infections of the trunk, legs, arms, and neck, excluding the feet, hands and groin. The causative agent is usually *T. rubrum* (incidence ranging from 32% to 60% of cases), followed by *T. tonsurans* (in 17.7–34.3% of cases).<sup>2,17</sup> Tinea corporis is more frequent in tropical and subtropical areas.<sup>29</sup> The infections are usually acquired by autoinoculation from other areas of the body such as the feet or scalp or by contact with animals.<sup>30</sup>

### Clinical features

Usually the patient presents with lesions on the body lasting from days to months with mild pruritus or even no symptoms. The typical presentation is an annular plaque that expands in a centrifugal pattern. The border is usually “active” with an erythematous elevated shape and occasionally small papules. The center of the lesion is paler (Fig. 24-3). Sometimes it presents with granulomatous plaques and is designated Majocchi’s granuloma. The lesions are more inflammatory if the cause is a zoophilic infection and usually present with vesiculation and crusted margins.<sup>30</sup>

### Differential diagnosis

The differential diagnoses include atopic dermatitis, allergic contact dermatitis, psoriasis, pityriasis versicolor, pityriasis alba, erythema migrans, subacute lupus erythematosus, annular erythema and mycosis fungoides.

### Treatment

Localized tinea corporis can be treated by topical imidazoles (e.g., clotrimazole), and butenafine or terbinafine.<sup>31,32</sup> Oral treatment is also useful as a short course of oral terbinafine.<sup>33</sup>

## Tinea cruris

The usual age of onset is adulthood and it affects more men than women. The causative organisms are *T. rubrum* followed by *T. mentagrophytes*.<sup>2</sup> Predisposing risk factors include a humid environment, warm and tight clothing, obesity and the application of topical corticosteroids. It can present simultaneously with tinea pedis or tinea unguium of the toenails.<sup>34</sup>

### Clinical features

The lesions are usually pruritic and localized in the groin area and thighs, and occasionally involve the buttocks. Large and well-demarcated red, tan or brown plaques with scaling can be seen. The margins are more elevated or active with some pustules or erythematous papules. As in other dermatophyte lesions, they follow a centrifugal pattern of dissemination.

### Differential diagnosis

The differential diagnoses include *Candida* intertrigo, inverse-pattern psoriasis, erythrasma and Langerhans cell histiocytosis.

### Treatment

Based on multiple randomized controlled trials, tinea cruris is best treated with a topical allylamine or a topical azole antifungal (highest strength of recommendation, A). The choice of treatment will depend on patient compliance, cost and convenience of administration. The fungicidal allylamines (naftifine and terbinafine) and butenafine (allylamine derivative) are the more expensive topical treatments. However, they are more convenient since the duration of treatment is shorter when compared with fungistatic azoles (clotrimazole, econazole, ketoconazole, oxiconazole, miconazole, and sulconazole).<sup>35</sup>

## Tinea pedis

Tinea pedis is more frequent in late childhood or young adults.<sup>1</sup> Its causative agent is *T. rubrum* (83% of cases),<sup>2,16</sup> especially in the interdigitale, dry, moccasin form, followed by *T. mentagrophytes* (moist or macerated form).<sup>8</sup> The duration varies from months to years since the majority of the infections are asymptomatic. This infection is related to humid and hot weather, use of occlusive footwear and marked sweating. It is more frequently found in individuals living in the countryside. This appears to be due to the difference in occupation between the rural population and city residents.<sup>34</sup>

### Clinical features

The clinical manifestations of tinea pedis vary according to the type of presentation. There are four types described.

- The interdigitale type, which can be dry or macerated. The dry presentation usually involves scaling and the macerated one includes peeling and fissuring of the toe web. The fourth and fifth interdigitale areas are the most commonly affected. However, it can spread to adjacent areas of the feet (Fig. 24-4).<sup>36</sup>
- The moccasin type is a well-demarcated erythematous plaque with small papules on the margin and scaling in the center; it usually affects the sole and the lateral borders of the feet.<sup>36</sup>





**Figure 24-4** Tinea pedis (courtesy of Adrina Motta MD, El Bosque University, Bogota, Colombia).



**Figure 24-5** Tinea unguium and tinea pedis due to *T. rubrum* (courtesy of Adrina Motta MD, El Bosque University, Bogota, Colombia).

- The inflammatory type is characterized by vesicles or small bullae filled with clear liquid affecting mostly the sole.
- The ulcerative type occurs when the interdigitale type extends into the dorsal and plantar surfaces of the feet.<sup>36</sup>

#### Differential diagnosis

The differential diagnoses include erythrasma, *Candida* intertrigo, psoriasis vulgaris, allergic contact dermatitis and dyshidrotic eczema.

#### Treatment

Treating tinea pedis with medications such as topical azoles (clotrimazole, ketoconazole, miconazole among others), allylamines (terbinafine, naftifine), benzylamines (butenafine) or hydroxypyridones (ciclopirox) is often sufficient. However, patients with large areas of involvement or with immunosuppression may benefit from an oral agent such as terbinafine, itraconazole, ketoconazole or fluconazole.<sup>37</sup>

### Tinea manum

This is a chronic, usually asymptomatic, dermatophytosis lasting from months to years. In the majority of cases the presentation is unilateral and affects mostly adults. The causative agent is usually *T. rubrum*.<sup>2</sup>

#### Clinical features

The lesions are usually erythematous, well-demarcated, scaling patches with hyperkeratosis on the palmar surfaces, sometimes with fissures.

#### Differential diagnosis

The differential diagnoses include conditions such as lichen simplex chronicus, atopic dermatitis and irritant contact dermatitis.

#### Treatment

The treatment of tinea manum is similar to that for tinea corporis or pedis. Topical or oral azole treatment is usually prescribed. The choice of medication depends on the severity of the clinical features and previous treatments.

### Tinea unguium

Tinea unguium or onychomycosis refers to fungal infection of the fingernails or toenails and is more frequent in adults.<sup>38</sup> It is common, responsible for up to 50% of diseases of the nail.<sup>39</sup> The most common agent is *T. rubrum*<sup>17,40</sup> which affects primarily the toenails, followed by *T. mentagrophytes*.<sup>39</sup> Meanwhile, *Candida* spp. are more frequent in the fingernails.<sup>17,18</sup> Other agents include (in minor percentage) non-dermatophyte fungi (moulds or yeasts).<sup>38,39</sup> The risk factors associated with onychomycosis include older age, immunodeficiency, genetic factors, abnormal nail morphology and previous *T. rubrum* infection. Recurrence is common in patients with these risk factors<sup>40</sup> or in those who are not compliant with treatment duration or if they receive inappropriate doses of medication.<sup>41</sup>

#### Clinical features

There are four types of onychomycosis: distal subungual, proximal subungual, white superficial, and candidal.<sup>38,42</sup> These types can be differentiated by their typical clinical presentation. Distal subungual onychomycosis is the most common type, representing approximately 58–85% of all cases.<sup>18,43,44</sup> In this type, the nail is friable and thickened with discoloration and subungual hyperkeratosis (Fig. 24-5).<sup>43</sup> In the proximal subungual type, the nail plate turns white proximally near the cuticle (this presentation is very common in immunosuppressed patients). In white superficial onychomycosis, the nails turn white and crumbly; in candidal onychomycosis, the nail plate separates from the nail bed.<sup>42</sup>

#### Diagnosis

The most sensitive and specific way to make the diagnosis of onychomycosis is by a PAS (periodic acid-Schiff) stain performed on a nail clipping. The nail samples should always be taken from the most proximal infected area. Culture is always mandatory.

#### Differential diagnosis

The differential diagnoses include lichen planus, nail psoriasis and yellow nail syndrome.<sup>42</sup>



## Treatment

Onychomycosis is a chronic and common infection of the nail that is often difficult to treat. The majority of clinical studies on onychomycosis have shown that the peak efficacy of the treatment is at week 48 or 52. Oral therapies such as terbinafine, itraconazole and fluconazole have been used with good results (particularly terbinafine and itraconazole<sup>45</sup>). The doses of these two medications may vary depending on the affected nail.<sup>37</sup> If it is a fingernail, the recommended treatment is given for months.<sup>46</sup> Some studies recommend combining oral and topical therapies (such as amorolfine and ciclopirox laquers), which appears to improve cure rates.<sup>47</sup>

## Scytalidium infections

*Scytalidium* spp. are dematiaceous or pigmented fungi that follow a similar path of infection as dermatophytes. The infection is acquired by contact with infected scales. It is not clear how these infections disseminate in the community and two types of organisms have been reported which include *Scytalidium dimidiatum* (a pigmented organism) and *Scytalidium hyalinum* (non-pigmented).<sup>48</sup>

*Scytalidium* spp. cause superficial infections similar to the ones caused by *T. rubrum*. Usually they are of the “dry type” which presents with scaling lesions located on the palms and soles. On occasion, the compromise is only unilateral and can affect the interdigital spaces of the feet. The nail compromise includes distal and lateral subungual onychomycosis, sometimes with a slight hyperkeratosis. It can also produce paronychia, when the infection extends upwards from the lateral nail border to the proximal nailfold.<sup>48</sup> In the literature, there are reports of subcutaneous infections such as mycetoma-like to disseminated disease, especially in severely immunosuppressed patients.<sup>49</sup>

The treatment response for *Scytalidium* infections is very poor, but conventional antifungal agents (e.g., terbinafine, azoles) are used as treatments.

## Candidiasis

*Candida* is one of the most frequently isolated yeasts in clinical practice.<sup>50</sup> This microorganism is part of the normal saprophytic flora colonizing mucosal surfaces and the skin. The majority of infections are caused by *Candida albicans*, although other species such as *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. guilliermondii* may be implicated in superficial infections.<sup>51,52</sup> Usually *C. albicans* infects patients with an underlying predisposition such as immunodeficiency and those who have received antibiotics (the use of antibiotics increases the vaginal and oral colonization by *Candida* species). A study in Australia showed that initial colonization by *Candida* spp. was present in 21% of women (95% confidence intervals (CI), 17–27%), rising to 37% (95% CI, 31–44%) after antibiotic treatment.<sup>53</sup>

### Clinical features

**Oropharyngeal candidiasis (Thrush).** Oropharyngeal candidiasis is seen more frequently in immunocompromised patients. It is very common in HIV patients, especially in those not receiving highly active antiretroviral therapy (HAART) (Fig. 24-6),<sup>51,54</sup> in whom the prevalence was as high as 90%.<sup>55</sup> Patients presenting with oral candidiasis may be asymptomatic or may complain of pain or a burning sensation in affected areas. The



**Figure 24-6** Oral candidiasis in a HIV patient (courtesy of Adrina Motta MD, El Bosque University, Bogota, Colombia).

lesions can occur anywhere on the oral mucosa (hard and soft palates, gums), the tongue, or extending back into the posterior pharynx.<sup>56</sup>

Oral candidiasis can have four clinical presentations: erythematous, pseudomembranous, hyperplastic, and angular cheilitis. The erythematous type is characterized by a flat red, subtle lesion or multiple lesions on the dorsal surface of the tongue and/or the hard/soft palates. Occasionally, the tongue is depapillated with red mucosal areas on its dorsal surface.<sup>57</sup> Pseudomembranous candidiasis appears as creamy white curd-like plaques on the tongue, buccal mucosa and other mucosal surfaces. The membranes can be wiped away, leaving a red or bleeding underlying surface. The lesions may be very small (1–2 mm) in size or cover the entire hard palate. The presentation of angular cheilitis is usually with fissuring and redness at either one or both corners of the mouth.<sup>57,58</sup> The hyperplastic type appears as a white patch on the commissures of the oral mucosa, and its location is usually the dorsal surface or the tongue.<sup>59</sup> Oral candidiasis can progress to esophageal and systemic candidiasis, depending on the immune system of the host.

**Vulvo-Vaginal Candidiasis (VVC) or vaginal thrush.** Vulvo-vaginal candidiasis affects immunocompetent women. It remains a common cause of morbidity, with nearly three-quarters of women affected during their lifetimes.<sup>53</sup> The infection can have an important physical and psychologic impact on women, affecting relationships with their partners.<sup>60</sup> Some studies have shown that VVC is caused by *C. albicans* in approximately 73%, with *C. glabrata* detected in around 20%.<sup>53</sup> The lesions characteristic of VVC are erythematous (sometimes whitish) plaques associated with soreness, irritation, discomfort and a creamy discharge.

**Candida intertrigo.** Infection of the skinfolds caused by *Candida* spp. is called intertrigo. It usually affects the skin of the groin and under the breast and armpits. Heat, hyperhidrosis and obesity are considered risk factors. The lesions are initially pustular on an erythematous base. Subsequently, the pustules erode and become confluent, forming erythematous, well-demarcated humid plaques surrounded by small pustules called satellite lesions.<sup>61</sup>

**Candidiasis interdigitale.** The lesions are very similar to those of intertrigo. The typical location is the interdigital spaces of the hands, particularly the third and fourth spaces (Fig. 24-7).



**Figure 24-7** Candidiasis interdigitale (courtesy of Adrina Motta MD, El Bosque University, Bogota, Colombia).



**Figure 24-8** Diaper dermatitis (courtesy of Adrina Motta MD, El Bosque University, Bogota, Colombia).

**Paronychia or candidal onychia.** This is involvement of the skin around the nail. The presentation is usually very painful with an erythematous, swollen plaque. It is common in persons who are in frequent contact with water. *Candida* is usually the etiologic agent, as in cases of chronic paronychia.<sup>62</sup>

**Diaper dermatitis.** The lesions of diaper dermatitis are very similar to intertrigo, but is localized in the perigenital and perianal areas, inner sides of the thighs and the buttocks. It is very common to find these lesions in small children related to the humidity of the diaper (Fig. 24-8).<sup>63</sup>

**Candidiasis of the nail.** Onychomycosis due to *Candida* is uncommon, except in patients with mucocutaneous candidiasis or persons who are in frequent contact with water. *Candida* onychomycosis can be divided into three categories.

- Infection beginning as paronychia (also called a “whitlow”), the most frequent type of *Candida* onychomycosis. The organism penetrates the nail plate only secondarily after it has involved the soft tissue around the nail. If the nail matrix becomes infected, transverse depressions (Beau’s lines) may appear in the nail plate and the persistent infection turns the nail convex, irregular, rough and finally dystrophic.
- *Candida* granuloma (incidence of <1% of onychomycosis cases). This usually appears in immunocompromised patients with chronic mucocutaneous candidiasis. The yeast invades the nail plate and may affect the entire thickness of the nail.
- *Candida* onycholysis occurs when the nail plate has separated from the nail bed. It is more frequently seen in the hands than the feet.<sup>64</sup>

**Mucocutaneous candidiasis.** Chronic mucocutaneous candidiasis is associated with primary T cell immune deficiencies and is characterized by persistent or recurrent *C. albicans* infections of the skin, nails, and mucous membranes without *Candida* sepsis (CMC).<sup>65</sup> The majority of patients have a selective defect of cell-mediated immunity against *C. albicans* which is demonstrated by cutaneous anergy to *Candida* antigen or decreased lymphoproliferative responses to *Candida* with normal immunoglobulins and antibody responses.<sup>66</sup>

#### Diagnosis

The diagnosis of *Candida* infections is made by direct microscopy which shows pseudohyphae and yeast forms which should be confirmed by culture.

**Table 24-1** Differential diagnosis of *Candida* spp. infections

Candidiasis	Differential Diagnosis
Oral candidiasis	Lichen planus, hairy tongue, lichen planus
VVC	Trichomoniasis, gardenella, lichen planus, lichen sclerosus et atrophicus, bacterial vaginosis
Intertrigo	Inverse-pattern psoriasis, erythrasma, dermatophytosis, pityriasis versicolor
Diaper dermatitis	Seborrheic dermatitis, atopic dermatitis, irritant dermatitis

#### Differential diagnosis

See Table 24-1.

#### Treatment

The azoles are the first line of therapy for *Candida* infections. Treatment with topical azoles (e.g., clotrimazole, miconazole) or intravenous azoles (i.e., ketoconazole, fluconazole, or voriconazole) is usually very effective. The dose may vary with the clinical presentation.<sup>46</sup> Due to the increased use of fluconazole, fluconazole-resistant candidiasis has emerged. The echinocandins (agents that inhibit glucan synthesis) are effective alternatives against fluconazole-resistant strains. These compounds have excellent clinical efficacy and provide improved safety profiles in individuals with superficial *Candida* infections.<sup>67</sup>

#### Diseases due to *Malassezia*

*Malassezia* is a dimorphic lipophilic yeast of the genus *Malassezia*, which colonizes the stratum corneum and forms part of the commensal flora of the skin.<sup>68</sup> This organism can produce different skin diseases such as tinea versicolor, seborrheic dermatitis and folliculitis.





**Figure 24-9** Tinea versicolor (courtesy of Adrina Motta MD, El Bosque University, Bogota, Colombia).

#### Tinea versicolor

Tinea versicolor is one of the most common benign recurrent hypopigmentary disorders around the world. It usually affects young adults or adolescents in tropical regions, and its prevalence is higher during the summer.<sup>69,70</sup> It is not contagious and the infection is due to the change to the mycelial phase of the dimorphic lipophilic yeast, *Malassezia* spp.<sup>71</sup> These organisms are located in body areas with abundance of sebaceous lipids such as the upper back, the trunk and the head.<sup>72</sup> The most common species involved in tinea versicolor include *M. globosa*, *M. sympodialis*, *M. furfur*,<sup>68,69</sup> and, less frequently, *M. obtuse*, *M. restricta* and *M. sloofiae*.<sup>73</sup>

**Clinical features.** The lesions are usually macules or slightly scaly, irregularly shaped patches with a variety of colors (white to pink or salmon to brown). The patches are commonly located on the trunk and back and are separated by areas of normal skin (Fig. 24-9).<sup>70</sup>

**Diagnosis.** The diagnosis is usually clinical but it can be confirmed by direct microscopy. The hyphae and blastocidia are characteristic.<sup>74</sup>

**Differential diagnosis.** The differential diagnoses include pityriasis alba, tinea corporis, psoriasis, seborrheic dermatitis and vitiligo.<sup>75</sup>

**Treatment.** The treatment usually involves topical agents (especially for children). The topical medications frequently used include selenium sulfide 2.5%,<sup>76</sup> clotrimazole 1%, bifonazole 1%, ketoconazole 2%,<sup>77,78</sup> and ciclopirox.<sup>79</sup> Oral medications are also available and include fluconazole single dose (400 mg),<sup>80</sup> itraconazole single dose (400 mg),<sup>80,81</sup> and ketoconazole.<sup>82</sup>

#### Seborrheic dermatitis

Seborrheic dermatitis is a common dermatosis affecting between 1% and 3% of the immunocompetent adult population.<sup>83,84</sup> The incidence of this disease is much higher in



**Figure 24-10** Seborrheic dermatitis (courtesy of Adrina Motta MD, El Bosque University, Bogota, Colombia).

immunocompromised patients, especially AIDS patients, ranging from 30% to 83%.<sup>85,86</sup> It is more common in infants during the first 3 months of life and in adults 30–60 years old.<sup>87</sup> The prevalence in children is around 10%.<sup>88</sup> The exact etiologic agent is not known and many factors have been suggested, including *Malassezia furfur* infection, hormone levels, nutritional deficits and other infections. The altered essential fatty acid pattern, increase of *M. furfur* colonization and inflammation have been the most extensively studied.<sup>83,87,89</sup>

**Clinical features.** The typical clinical presentation is greasy scaling of the scalp with erythema. Compromise of the nasolabial folds and the postauricular skin can also be seen (Fig. 24-10). Usually the lesions present at areas of increased sebaceous gland activity such as the auricles, eyebrows, trunk and bearded area.<sup>87</sup> Patients commonly report a pruritic sensation. In children, the clinical presentation is characterized by thick, greasy scales on the vertex of the scalp.<sup>90</sup> The diagnosis is usually clinical.

**Differential diagnosis.** The differential diagnoses include atopic dermatitis, dermatophytosis, psoriasis, rosacea, systemic lupus erythematosus and candidiasis.<sup>87</sup>

**Treatment.** The therapies for seborrheic dermatitis include antifungals, keratolytic agents, antiinflammatory and immunomodulatory agents. The antiinflammatory/immunomodulatory agents include steroid shampoos, topical steroids (such as fluocinolone, betametasone valerate and desonide) and topical calcineurin inhibitors (tacrolimus ointment or pimecrolimus cream). The keratolytic agents more frequently used are salicylic acid shampoo, tar shampoo and zinc pyrithione shampoo. The antifungal therapies used with a good rate of response include ketoconazole shampoo, ciclopirox and selenium sulfide shampoo.<sup>87</sup>

#### *Malassezia* folliculitis

*Malassezia* folliculitis is more common in the subtropical and tropical regions. Clinically, it appears as a pruritic, monomorphic eruption of papules and pustules on the trunk (particularly the back). It may affect the upper arms and occasionally the neck and face.<sup>91</sup> It is more frequent in severely ill patients or after sun exposure. The treatment involves oral itraconazole or ketoconazole.<sup>91</sup>



## Miscellaneous superficial fungal infections

### Piedra

Piedra is an asymptomatic infection of the hair shaft<sup>92</sup> also known as trichomycosis nodularis. It affects both sexes and all ages. White piedra is more common in temperate and semi-tropical climates (South America, India, Africa, Japan, Middle East and southern USA). Black piedra is more frequent in the tropics worldwide.<sup>72</sup> The etiologic agents of black and white pedras are *Piedraia hortae* and *Trichosporon beigeli* (also, *T. inkin*, *T. asahii* or *T. mucoides*), respectively.<sup>72</sup>

**Clinical features.** Black piedra is characterized by darkly pigmented nodules in the hair shaft that vary in size (up to a few millimeters in diameter). The lesions produce a metallic sound when brushing the hair. The nodules are hard and firmly attached to the scalp hair, especially the frontal area.<sup>72</sup> White piedra affects the pubic and axillary hair, moustaches, eyebrows and eyelashes. It appears as lightly pigmented, white to brown nodules with a soft texture, loosely attached to the hair shaft.

**Diagnosis.** The diagnosis is made with conventional methods such as direct microscopy with KOH solution, and fungal stains such as chlorazol black E stain or Parker blue-black ink.

**Differential diagnosis.** Includes pediculosis capitis, pediculosis pubis, tinea capitis, moniletrix, and trichorhexis nodosa.

**Treatment.** The recommended treatment is cutting or shaving the hair involved with black or white piedra. White piedra can also be treated with topical antifungals such as ciclopirox, selenium sulfide 1–2.5%, pyrithrione zinc 2–10%, or imidazoles.<sup>72</sup>

### Tinea nigra

Tinea nigra is a superficial mycosis of the stratum corneum caused by traumatic inoculation from soil, wood or compost.<sup>72</sup> It is common in tropical regions of Central and South America, Africa and Asia.<sup>72</sup> It is more common in females and the infection is most evident in young people and children.<sup>72,92</sup> Tinea nigra appears in the body areas with increased concentration of eccrine sweat glands. The causative fungus is *Exophiala werneckii*.<sup>72</sup>

**Clinical features.** Characterized by single discrete and painless oval macules or patches with light brown to black color usually located on the palms or soles (Fig. 24-11). Occasionally the lesion involves the fingers and nails (Fig. 24-12). It grows slowly and can have a prolonged course (years).<sup>92</sup>

**Diagnosis.** The diagnosis is made by scraping the stratum corneum with a scalpel and performing KOH microscopic examination of the samples. It should be confirmed by culture.

**Differential diagnosis.** The differential diagnosis includes melanocytic nevus, dysplastic nevus, pinta, syphilis and Addison's disease.

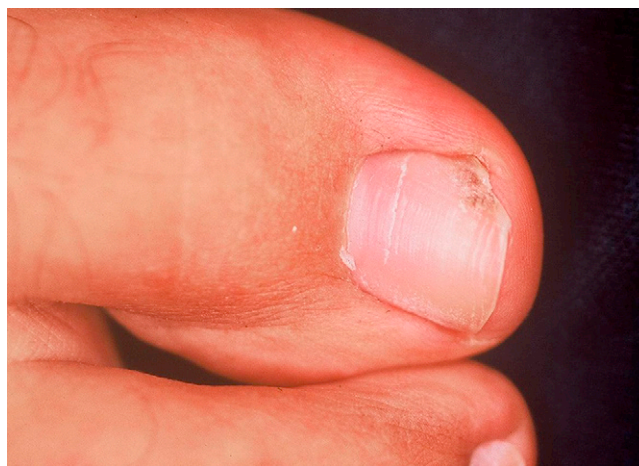
**Treatment.** The treatment alternatives include oral ketoconazole, itraconazole, and miconazole. Topical therapies include retinoic acid, ciclopirox, and terbinafine.<sup>37</sup>

### Alternariosis

Alternariosis is a form of phaeohyphomycosis (black moulds) caused by cosmopolitan opportunistic moulds of the genus *Alternaria*.<sup>93</sup> It may present as localized epidermal eczema-like lesions with erythema and desquamation or with red papules and a granular surface. There may also be chronic



**Figure 24-11** Tinea nigra (courtesy of Adrina Motta MD, El Bosque University, Bogota, Colombia).



**Figure 24-12** Tinea nigra (nail) (courtesy of Adrina Motta MD, El Bosque University, Bogota, Colombia).

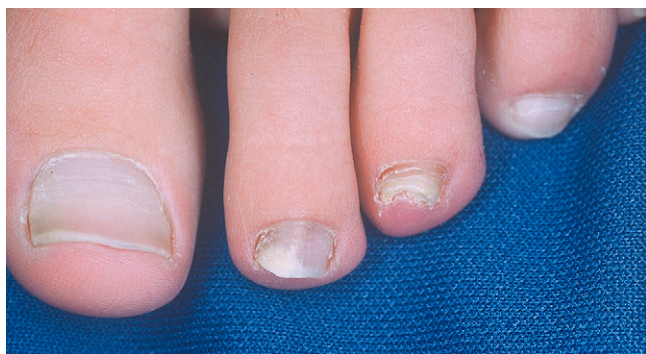
solitary verrucous lesions of the skin, which occasionally form abscesses. The diagnosis includes direct microscopy and culture. Histopathology studies are required in specific circumstances.<sup>93</sup>

This infection is usually asymptomatic and patients do not notice the skin lesions. The lesions can last weeks or months. Fungal spread from primary cutaneous lesions has not been described.<sup>93</sup>

The treatment includes surgical excision or antifungal medications such as ketoconazole, itraconazole, voriconazole, and sertaconazole.

### Fusariosis

Fusariosis is caused by opportunistic moulds of the genus *Fusarium*, present in soil and plants. The majority of infections are superficial in patients with good immune status<sup>94</sup> and the clinical presentations include interdigital intertrigo and paronychia (Fig. 24-13).<sup>93</sup> The diagnosis is made by direct microscopy and culture. In certain cases, histopathologic studies are required.



**Figure 24-13** Onychomycosis due to *Fusarium* (courtesy of Adrina Motta MD, El Bosque University, Bogota, Colombia).

The treatment includes surgical excision and antifungal drugs such as amphotericin B, voriconazole, sertaconazole, and terbinafine.<sup>93</sup>

#### Otomycosis

The term “otomycosis” is used to describe mould or yeast infections of the external auditory canal. The *Aspergillus* spp. represent almost 95% of the mould isolates. *Aspergillus niger* is the most frequent species, followed by *A. fumigatus* and *A. flavus*. The infections are not contagious.<sup>95</sup> The clinical presentation may vary with chronic, acute invasive or chronic invasive forms described. The diagnosis is reached by direct microscopy and culture. Histopathology is required in some cases. The prognosis in immunocompetent patients is good, but immunocompromised patients can develop acute invasive or chronic invasive forms that can be life threatening.

Treatment includes debridement and cleansing of the affected areas in combination with topical antifungal agents such as nystatin, clotrimazole, ciclopirox, imidazole or amphotericin B.<sup>95</sup>

## Subcutaneous mycosis

### Mycetoma

Mycetoma is a chronic infection of the skin and subcutaneous tissue caused by fungi (eumycetoma) and bacteria (actinomycetoma).<sup>96</sup> Although the term “mycetoma” means a “fungal tumor,” bacteria are the most common causes of this infection.<sup>97</sup> Actinomycetoma have a worldwide distribution, especially in tropical countries with savannahs or forests, such as Central America, Mexico, Venezuela, Brazil, Africa, the Middle East, India, Pakistan, and Bangladesh. The organisms causing mycetomas can be found in the soil and plants<sup>96</sup> and the most frequently isolated species of Eumycetoma around the world is *Madurella mycetomatis*, particularly in Africa and India. In the United States, *Pseudallescheria boydii* is the predominant species. Other organisms associated with eumycetoma include *Madurella grisea*, *Leptosphaeria senegalensis*, and *Scedosporium apiospermum*. Actinomycetoma are frequently

seen in Mexico and Central America, with *Nocardia brasiliensis*, *Streptomyces somaliensis*, *Actinomadura madurae*, and *Actinomadura pelletierii*<sup>96,97</sup> amongst the most common bacterial species. Mycetoma usually affect adults (20–40 years old) with a male predominance (3.7:1) which is attributed to the risk of exposure to organisms in the soil during outdoor activities.<sup>97</sup>

#### Clinical features

The organism is usually implanted into the host tissue after a penetrating skin injury by trauma (stones, splinters and thorns) and may persist for many years without clinical signs of infection. The mechanisms by which the organisms evade the host defenses are unknown but may be related to the ability to adapt and survive adverse environmental conditions by producing certain proteins (e.g., melanin) or changing the cell metabolism (e.g., thickening the cell wall). The host's inflammatory response is mostly neutrophilic and associated with the presence of some epithelioid cells, plasma cells, lymphocytes, and giant cells. Inside the inflammatory mass the fungal grains (or mycetoma grains) can be found with a diameter of up to 5 mm. The organisms usually spread only to adjacent tissue but in certain cases, they have also been found to involve the lymph nodes (*Streptomyces somaliensis*). The lesion grows slowly and tends to coalesce into a larger area which is usually painless with tumefaction, with a darker aspect and firmer consistency than the surrounding skin.<sup>96,97</sup>

The actinomycetoma grows faster than the eumycetoma, the lesion is more destructive with more inflammation and early bone involvement. Also, the actinomycetoma tends to be more suppurative and with more inflammation which may lead to deformity and disability of the affected limb. The lesions can become painful at late stages when the nerves are inflamed or destroyed. Nonetheless, some authors suggest that the organisms involved in mycetoma produce anesthetic substances which prevent the development of painful stimuli.<sup>97</sup> Conversely, the eumycetoma grow at a slower rate, producing lesions with defined margins, remaining encapsulated for long periods and exhibiting a fibrotic pattern (Fig. 24-14). Both eumycetoma and actinomycetoma produce nodules, abscesses and fistulae with drainage of viscous or purulent exudate. Occasionally, the granules full of microorganisms can be detected with the naked eye.

#### Diagnosis

Examination of the grains is of paramount importance for the diagnosis of mycetoma. The macroscopic observation of the aspect, shape and color of the grain may help to identify the possible microorganism involved. For example, white grains are produced commonly by *Pseudallescheria boydii*, *Acremonium* spp., *Nocardia asteroides* and *N. brasiliensis*. Black grains are associated with the presence of *Madurella mycetomatis*, *M. grisea* and *Leptosphaeria senegalensis*. Bacterial species such as *Actinomyces israelii* and *Actinomadura madurae* tend to produce white to cream grains.<sup>97</sup> Additionally, microscopic examination of the grains should be performed to seek for hyphae, filaments or any other fungal or bacterial element. Culture of the lesion should be performed to isolate and identify the causative organism. Computed tomography (CT) and ultrasound examinations are helpful in defining the extent of the lesion and soft tissue involvement.





**Figure 24-14** Eumycetoma (courtesy of Daniel Carrasco MD, Dermatology Association of Texas, USA).

### Differential diagnosis

The differential diagnoses include chronic osteomyelitis of other causes, tuberculosis, atypical mycobacterial infections, botryomycosis, Kaposi's sarcoma, acral melanoma, actinomycosis,<sup>97</sup> blastomycosis, chromoblastomycosis and foreign body granuloma.

### Treatment

Actinomycotic mycetoma may respond to prolonged medical treatment with antibiotics and other chemotherapeutic agents, including streptomycin combined with either dapsone or trimethoprim-sulfamethoxazole. The cure rates vary from 60% to 90%.<sup>97</sup> Surgery is indicated in cases refractory to medical treatment. Eumycetoma rarely respond to medical therapy but some cases (particularly those caused by *Madurella mycetomatis*) may respond to ketoconazole, itraconazole or voriconazole.<sup>98</sup>

## Sporotrichosis

Sporotrichosis is a subcutaneous or systemic fungal infection with a global distribution (the majority of cases reported in the Americas, Australia, Asia and Africa). It is caused by *Sporothrix schenckii*, a saprophytic microorganism that can be found as a mould in wood, vegetable matter or soil in humid climates. It usually affects more males than females and tends to present in patients with certain occupational exposures such as agricultural workers, farmers, gold miners, laboratory workers and florists. The infection starts after traumatic inoculation of the microorganism into the skin through thorns or other plant matter. Subsequently, the microorganism spreads through the lymph nodes and in rare cases to subcutaneous tissues.<sup>97,99</sup>

### Clinical features

After inoculation into the skin, *S. schenckii* grows locally and can be limited to the site of the inoculation or may extend along the proximal lymphatics. After an incubation period of approximately 3 weeks, the patient develops asymptomatic lesions with no signs of systemic acute infection (usually afebrile).

Sporotrichosis can present in three forms: plaque (Fig. 24-15), lymphangitic or disseminated. The plaque sporotrichosis initiates as a subcutaneous papule or nodule, which appears



**Figure 24-15** Sporotrichosis.

after the inoculation. The borders are irregular and the color varies from pink to purple (Fig. 24-16). Verrucous plaques, indurated or crusted ulcers may occur.<sup>97,99</sup> The lymphangitic form is characterized by lymphatic extension to the local skin area (Fig. 24-17). In this presentation, the lymphatics become indurated, thickened and nodular.<sup>99</sup> In the disseminated type, hematogenous dissemination involves the skin (multiple widespread nodules and ulcers), lungs, joints, eyes and even meninges.

### Diagnosis

The diagnosis is made by clinical suspicion and isolation of the microorganisms on culture. The skin biopsy usually shows Langerhans-type giant cells, microabscesses and, in 40% of cases, the "sporothrix asteroid body" (which consists of a central yeast with radiating eosinophilic spicules) can be found. The sporotrichin skin test detects a delayed-type hypersensitivity reaction and is useful as an adjuvant diagnostic tool.<sup>99</sup>

### Differential diagnosis

The differential diagnosis includes tularemia, cat-scratch disease, cutaneous tuberculosis, primary syphilis, bacterial pyoderma, blastomycosis, chromomycosis, mycetoma and leishmaniasis.

### Treatment

The treatment involves long courses of antifungal agents such as itraconazole, fluconazole, ketoconazole and terbinafine. Also, a saturated solution of potassium iodide could be used for at least 4 weeks after apparent clinical "cure."

## Chromomycosis

Chromomycosis is a subcutaneous chronic fungal infection caused by different species of dematiaceous fungi. These organisms are saprophytic fungi found in soil, rotten palm tree trunks, wooden fence posts and wood. Five species cause the majority of the disease.

- *Fonsecaea pedrosoi* is the most common in tropical regions with high humidity and rainfall rates.
- *Cladosporium carrioni* is frequent in tropical countries with semi-arid regions and little precipitation such as Cuba, Venezuela, Australia and South Africa.<sup>100</sup>





**Figure 24-16** Sporotrichosis.



**Figure 24-17** Sporotrichosis (courtesy of Adrina Motta MD, Universidad El Bosque, Bogota, Colombia).

- *Phialophora verrucosa*.
- *Fonsecaea compacta*.
- *Rhinocladiella aquaspersa*.

Other species include *Wangiella dermatitides*, *Cladophiala phora ajelloi*, *Taniolella bopii*, and *Exophiala spinifera*.<sup>97,100</sup>

In general, chromomycosis is more common in tropical and subtropical America (Mexico, Costa Rica, Puerto Rico, Cuba, Colombia, Brazil and Ecuador) and Africa.<sup>100</sup> It is also more frequent in men than in women and it usually affects workers in rural areas.<sup>96</sup> The organism enters and penetrates the skin after local trauma. The local host reaction involves predominantly a hyperplastic inflammatory response that leads to a chronic granulomatous inflammatory process with pseudoepitheliomatous hyperplasia.<sup>96,97,101</sup>

#### Clinical features

The initial lesion is a single nodule with scaling at the site of inoculation. After months or years, new nodules and verrucous plaques appear with islands of normal skin between them. Subsequently, the lesions become large with a cauliflower aspect and “black dots” of hemopurulent material that bleed easily due to their friable granulation tissue (Fig. 24-18). Occasionally, the lesions can be large (10–20 cm in diameter) with lymphatic compromise (elephantiasis).<sup>96,97</sup>



**Figure 24-18** Chromomycosis.

#### Diagnosis

The diagnosis is performed by biopsy. The characteristic histopathologic feature is the presence of the muriform cells (dark-walled polyhedral structures), known as Medlar bodies, which are suggestive of chromomycosis. Cultures are useful in identifying the causative species but the yield is poor.<sup>97</sup>

#### Differential diagnosis

The differential diagnoses include verrucous leishmaniasis, sporotrichosis, lobomycosis, verrucous tuberculosis, and verrucous carcinoma.<sup>96,97</sup>

#### Treatment

The treatment is difficult and the response to antimycotic drugs is limited.<sup>101</sup> Surgical treatment is still the best choice to manage this disease. The combination of terbinafine and itraconazole for long periods of time has been reported to be partially effective in certain cases.<sup>97</sup>

### Rhinosporidiosis

Rhinosporidiosis is a chronic granulomatous disease caused by *Rhinosporidium seeberi*. This infection is characterized by chronic and benign polyps that affect mostly the mucous membranes (nostrils and conjunctiva). The taxonomy of this organism has been controversial, with investigators reporting it as a fungus, but it is now considered to be a protozoan parasite. Therefore, we are including it for historic reasons.<sup>97</sup> This infection has a worldwide distribution including India, Sri Lanka, Africa and South America. In countries such as Brazil, Venezuela, Colombia and Paraguay, the cases tend to be limited to specific areas.<sup>100</sup> The transmission is thought to be through dust and water, in view of the high frequency among sand collectors and men who work in rivers or lakes.<sup>97,100</sup> The infection affects mostly males between the ages of 20 to 40.<sup>97,100</sup>

### Clinical features

The affected patient usually develops pedunculated, unilateral, polypoid masses in the nasal cavity (inferior cornet, septum, nasal floor, middle cornet, meatus and nasal roof) which can be lobulated and confluent. The surface of the lesion is commonly friable with white spots (sporangia), exhibiting irregular borders which bleed easily,<sup>97,100</sup> predisposing to nasal discharge and pruritus. The ocular lesions have similar presentations, affecting mainly the conjunctiva and the lacrimal sac. Some other affected areas include the pharynx, palate, larynx, trachea, bronchia and esophagus. Lesions in the upper respiratory passages or gastrointestinal tract may lead to obstruction, cough, hemoptysis, or painful swallowing.<sup>100</sup>

### Diagnosis

The diagnosis is made by biopsy with demonstration of thick-walled giant sporangia.<sup>97</sup>

### Differential diagnosis

The differential diagnoses include coccidioidomycosis, myospherulosis and pyogenic granuloma.

### Treatment

The treatment includes surgery plus local injection of amphotericin B. Recurrences are common.<sup>97</sup>

## Zygomycosis

Zygomycosis is a life-threatening fungal infection that occurs in immunocompromised patients. It is caused by fungi of the class Zygomycetes. This infection can be divided into at least six clinical categories: rhinocerebral, pulmonary, cutaneous, gastrointestinal, disseminated, and miscellaneous. In the cutaneous presentation, the organisms are usually inoculated into the skin as a result of traumatic implantation of soil, maceration of skin by a moist surface or through intravenous catheters or subcutaneous injections.<sup>102</sup> The most important risk factors for cutaneous zygomycosis are burns, traumatic disruption of skin, and maceration of the skin allowing the organisms to penetrate into deeper tissues.

Two specific forms of subcutaneous infection can be seen and occur sporadically.

### Subcutaneous zygomycosis due to *Basidiobolus*

This form of zygomycosis usually causes a firm subcutaneous infiltration, often on the proximal parts of the limbs, and affects more children and adolescents than adults (especially in Africa, India, Middle East, Asia and Europe).

**Clinical features.** *Basidiobolus* infection affects mostly the shoulders, pelvis and hips or the proximal part of the limbs. It is characterized by rubbery, painless masses, sometimes with ulcerations on the surface. Deep invasion is uncommon, but cutaneous disease can be very invasive locally and penetrate from the cutaneous and subcutaneous tissues into the adjacent fat, muscle, fascia, and even bone.<sup>102</sup>

**Diagnosis.** The diagnosis is made by biopsy demonstrating the typical fungal elements. The biopsy is characterized by inflammation with eosinophils and thick aseptate hyphae. *Basidiobolus* can be cultured in vitro to confirm the diagnosis.

**Treatment.** The treatment includes itraconazole for several months or saturated solution of oral potassium or trimethoprim-sulfamethoxazole.

### Subcutaneous zygomycosis due to *Conidiobolus*

This type of zygomycotic infection is due to *Conidiobolus coronatus*, which can be found as a saprophytic fungus of leaves and plants in tropical environments (West Africa, India and Latin America). It is confined to the facial area and affects mostly adult men.

**Clinical features.** The lesions may be asymptomatic and they are not easily noticed until unilateral nasal obstruction is present. When symptomatic, the affected area is swollen and involves the nasal bridge and the upper and lower face.

**Diagnosis.** The clinical appearance is typical. The biopsy confirms the diagnosis and the findings are similar to those specified for *Basidiobolus*. Microbiologic cultures are the key for the diagnosis.

**Treatment.** The treatment is similar to that described for *Basidiobolus* infections. A recently approved azole, posaconazole, has been used with a success rate of 54%.<sup>103</sup>

## Phaeohyphomycosis

Phaeohyphomycosis is caused by a heterogeneous group of darkly pigmented (dematiaceous or pheoid) fungi widely distributed in the environment which occasionally cause infection in humans.<sup>104</sup> These black moulds may affect the layers of the epidermis (tinea nigra), dermal tissues (alternariosis) and deep dermal or subcutaneous structures. The main organisms involved are *Exophiala jeanselmei*, *Wangiella dermatitidis* and *Bipolaris* spp. However, more than 20 other fungi have been cited as etiologic agents of these infections. The route of inoculation and infection is controversial.

### Clinical features

Subcutaneous phaeohyphomycosis usually presents as nodules or erythematous plaques without symptoms or signs of systemic involvement.

### Diagnosis

Diagnosis is based on histopathologic examination of excised cysts, which shows an inflammatory cyst with a fibrous capsule and a granulomatous reaction with some neutrophils and epithelioid cells containing hyphal elements. The Masson-Fontana stain for melanin is usually used. The organisms can be cultured and identified.

### Treatment

The treatment of choice is surgical. Itraconazole and posaconazole have been used with relative success. Early experience with posaconazole has yielded encouraging results with a cure rate of about 80%.<sup>103</sup>

## Lobomycosis

Lobomycosis is an uncommon and chronic subcutaneous mycosis characterized by numerous nodular lesions reminiscent of keloids. It is caused by *Lacazia loboi* whose natural reservoir is unknown. The infection is restricted to the Amazon rainforest area in South America (Brazil, Colombia, Ecuador, Bolivia, Peru, Guayana and Surinam) and there are sporadic cases reported in Mexico and Central America.<sup>97</sup> The soil and vegetation seem to be the source of the organism since the infection affects humans who live in rural areas. The fungi are

introduced directly into the skin through a penetrating injury with a thorn prick or insect bite.<sup>97</sup>

### Clinical features

The lesion commences as a papule after inoculation with associated pruritus or a burning sensation. Occasionally, the lesion regresses and leaves a scar, but it never fully disappears. After several months or years the typical keloid-like lesion appears, which is usually a solid and smooth nodule with brown to pink color varying in size. The most common affected areas are the ear lobes as well as the upper and lower extremities (see Fig. 24-18). The lesions on soles and palms may be verrucous and may have features similar to those of chromomycosis.<sup>100</sup>

### Diagnosis

Diagnosis is made by biopsy or direct examination of the skin lesions with the identification of fungal elements in the tissue.

### Differential diagnosis

The differential diagnoses include lepromatous leprosy, keloids, xanthoma and dermatofibrosarcoma protuberans.

### Treatment

For small lesions electrosurgery, cryosurgery or surgical excision may be curative. For extensive lesions, surgical debridement is the treatment of choice, although high rates of relapses can be seen. Treatment with clofazimine, sulfonamides, ketoconazole, amphotericin B and flucytosine has been used without much success.<sup>100</sup>

## Conclusion

The cutaneous mycoses are common diseases affecting people worldwide. The spectrum of clinical manifestations varies according to the organism involved and the infection site. In general, fungi affecting the superficial layers of the skin are endemic in many parts of the world and disseminate by direct contact. The subcutaneous mycoses are usually the result of inoculation of fungi in deeper layers of the skin and subcutaneous tissues. The immune status of the individual plays a significant role in controlling these infections. Particularly, patients with defects in the cellular immune responses are predisposed to disseminated and life-threatening presentations of a variety of fungal infections where the portal of entry is the skin.

The antifungal armamentarium has grown significantly in the last decade; new azole compounds, echinocandins and lipid preparations of polyenes provide effective new alternatives for the treatment of these infections.

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# Fungal infections of bone and joint

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Fungal infection of the musculoskeletal system is a challenging but uncommon clinical problem that often eludes detection, especially in patients with an isolated focus of disease, although the cause may be obvious in a patient with overt infection elsewhere. Skeletal fungal infection most often results from the hematogenous dissemination of a fungal organism from a primary source of infection (usually pulmonary). The presence of a foreign body, such as a joint prosthesis, may predispose to certain fungal infections, especially by *Candida* species. The joint space may also become infected as a result of extension from an adjacent focus of osteomyelitis. In some instances, however, joint or tendon sheath infection and, to a lesser degree (except in areas of the world where mycetomata are prevalent), bone infection may occur as the result of direct inoculation of the organism in the setting of trauma, surgery, arthrocentesis, or therapeutic joint injection. The development of arthritis in a patient with a pulmonary fungal infection (e.g., coccidioidomycosis, histoplasmosis, and blastomycosis) may also be the result of a systemic immunologic response rather than the presence of the pathogen within the intraarticular space.

## Epidemiology

Virtually all of the several hundred fungi pathogenic in humans have been reported to cause musculoskeletal infection (Tables 25-1, 25-2).<sup>1-5</sup> The frequency with which arthritis or osteomyelitis occurs, the clinical presentation, and the outcome vary, depending on the specific fungal agent and on differences among hosts. Thus, although fungal arthritis or osteomyelitis due to the endemic dimorphic fungi, such as *Coccidioides* spp., *Blastomyces dermatitidis*, and *Histoplasma capsulatum*, often occur as a result of hematogenous dissemination of infection in patients without overt immunodeficiency,<sup>1,4-7</sup> patients with hematologic malignancy, bone marrow and solid organ transplant recipients who receive immunosuppressive therapies, and patients receiving long-term corticosteroids are especially at risk for skeletal infection by several other fungi.<sup>8-10</sup> Either reactivated or severe acutely disseminated fungal infection has been reported in persons receiving treatment with TNF- $\alpha$  inhibitors and human interleukin-1 receptor antagonists, including infection due to *H. capsulatum*, *Candida*, and *Aspergillus* but also *C. immitis* and *Cryptococcus*.<sup>11</sup> Although patients with AIDS are especially vulnerable

to disseminated infection due to *Cryptococcus*, *C. immitis*, and *H. capsulatum*,<sup>9</sup> children with chronic granulomatous disease are at risk for osteomyelitis due to both *Candida* and *Aspergillus*.<sup>8</sup> And although neonates with candidemia are at high risk for joint space infection, *Candida* spp. are rarely the cause of musculoskeletal infection in adults and are found almost exclusively in individuals with readily apparent predisposing factors, such as those with indwelling central venous catheters (often in association with the administration of long-term antibiotic therapy and parenteral nutrition), those undergoing hemodialysis, and injection drug abusers.

## Pathogenesis

The pathogenesis of fungal osteomyelitis has not been investigated but presumably is similar to that observed with bone infection of bacterial origin. Fungal organisms that reach the synovium through the bloodstream form granulomas (e.g., coccidioidomycosis) or microabscesses (e.g., candidiasis) and subsequently infect the synovial fluid. In cases of inoculation directly into synovial fluid, the organisms are presumably phagocytized by both professional phagocytes and synovial lining cells and proliferate within the synovium. The resultant inflammatory response produces an exudative joint effusion. Release of enzymes such as collagenase and other proteolytic enzymes may damage joint surfaces. In cases of chronic granulomatous synovitis, such as in coccidioidomycosis, exuberant synovial proliferation occurs, and the resultant pannus may erode articular cartilage and even subarticular bone (Fig. 25-1A,B).

## Clinical features

### Bone disease

When fungal osteomyelitis is the result of hematogenous dissemination, bone disease may represent only one portion of a multisystemic illness, or it may be an isolated clinical problem (see Table 25-2). The most common complaint is local pain. Although often indolent in its evolution, some cases are more acute with erythema, swelling, and tenderness. “Cold” soft tissue abscesses and sinus tracts may be seen in chronic infections, particularly in coccidioidomycosis and blastomycosis (Fig. 25-2).



**Table 25-1** Approximate incidence of osteomyelitis and joint involvement in fungal infection

Agent	Acute primary infection	Disseminated infection	
	Aseptic arthritis	Osteomyelitis	Joint infection
<i>Blastomyces dermatitidis</i>	Unusual	7–48%	2.5–8%
<i>Coccidioides immitis</i>	3–5%	10–42%	25–30%
<i>Cryptococcus neoformans</i>	—	3.5–5%	<1%
<i>Candida species</i> *	—	15.4%	DNA <sup>†</sup>
<i>Histoplasma capsulatum</i> ‡ <sup>§</sup>	1.6%	Rare	Rare
<i>Paracoccidioides brasiliensis</i>	—	<5%	DNA <sup>†</sup>
<i>Sporothrix schenckii</i>	—	DNA <sup>†</sup>	0.03%

\*Percentage reflects the approximate incidence of disease in patients who are at risk and have documented fungemia; joint space infection is common in neonates with disseminated candidiasis.

†Data not available (but unusual).

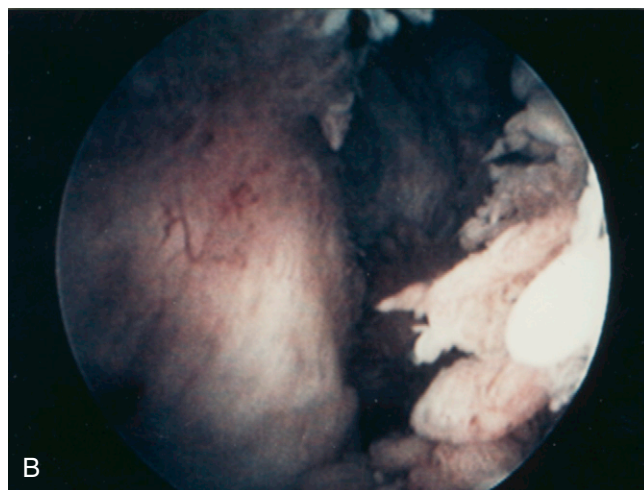
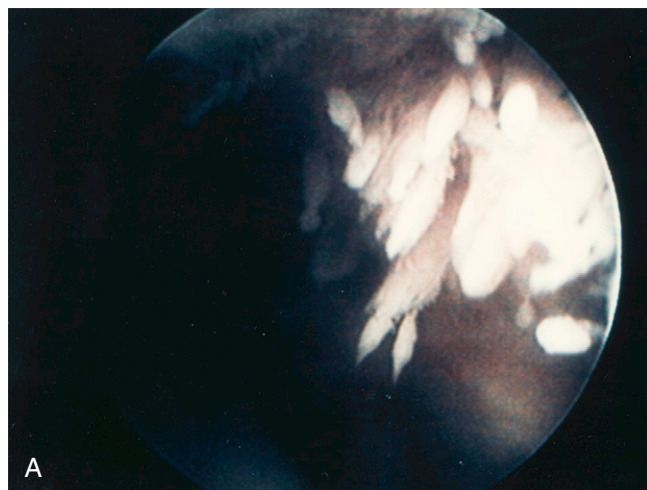
‡Bone marrow involvement occurs in more than 90% of patients with disseminated histoplasmosis.

§Bone involvement occurs in up to one half of patients with disseminated infection due to *Histoplasmosis capsulatum* var. *duboisii*.

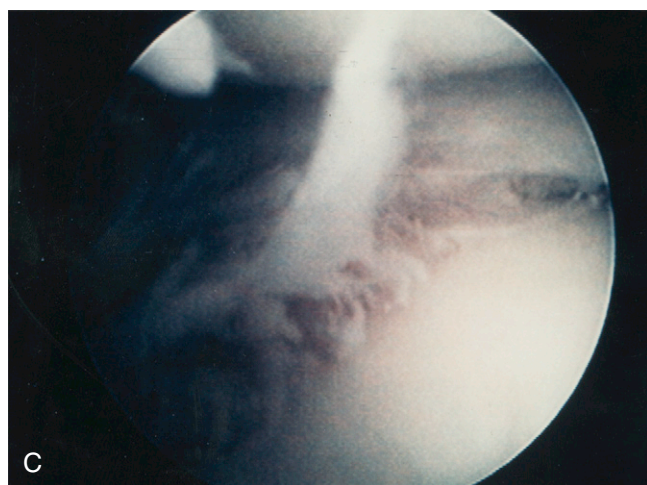
**Table 25-2** The endemicity and epidemiology of fungal skeletal infection

Organism	Endemicity	Host risk factors	Mode of infection
<i>Candida species</i>	Normal human commensal	Hematologic malignancy, indwelling catheters, long-term antibiotic use, high-risk neonates	Hematogenous, rarely direct inoculation from trauma or injection
<i>Coccidioides spp.</i>	Arizona, New Mexico, California, Nevada, western Texas, northern Mexico, others	Often immunocompetent host (50%); diabetes, renal failure, corticosteroids	Hematogenous
<i>Blastomyces dermatitidis</i>	Ohio, Missouri, Mississippi River Valleys, Southeastern United States, Africa, Middle East	Usually immunocompetent host (>75%–94%); diabetes, alcoholism, corticosteroids, malignancy, organ transplantation	Hematogenous, rarely direct inoculation
<i>Sporothrix schenckii</i>	Worldwide	Alcoholism, diabetes (80%); rarely immunocompromised	Hematogenous, may be direct inocula
<i>Histoplasma capsulatum</i>	Ohio, Missouri, Mississippi River Valleys, Central and South America, others	Usually immunocompromised (e.g., AIDS, lupus); occasionally immunocompetent host	Hematogenous
<i>Cryptococcus neoformans</i>	Worldwide	Organ transplantation, AIDS, hematologic malignancy, diabetes, corticosteroids	Hematogenous
<i>Paracoccidioides brasiliensis</i>	Central and South America	Immunocompetent host	Hematogenous

Modified from Kemper CA, Deresinski SC: Fungal arthritis. In Maddison PJ, Isenberg DA, Woo P, Glass DN (eds): Oxford Textbook of Rheumatology. Oxford University Press, Oxford, United Kingdom, 1993.



**Figure 25-1** Proliferative synovitis due to *Coccidioides immitis*, with fronds of tissue extending into the joint space visualized on arthroscopy of an infected knee joint. (Courtesy of Michael F. Dillingham MD).



**Figure 25-2** (A) Chronic coccidioidal arthritis demonstrating the right elbow joint fixed in flexion. (B) The sinus tracts intermittently drain material from which *Coccidioides immitis* is recoverable in culture. (Courtesy of John S. Hostetler MD).

Radiographs reveal lytic lesions with little new bone formation, at least initially. The differential diagnosis on the basis of clinical and radiographic disease is lengthy and includes tuberculosis, sarcoidosis, osteogenic sarcoma, Ewing's sarcoma, malignant metastasis, actinomycoses, Langerhans cell histiocytosis, and, possibly, osteomyelitis due to such

organisms as *Staphylococcus aureus* and *Salmonella* species.<sup>9,12</sup> Many of these may be distinguished from fungal osteomyelitis on the basis of a periosteal reaction and new bone formation. Histologic evidence of necrotizing granulomas may suggest, in addition to a fungal infection, the presence of tuberculosis or chronic osteomyelitis due to *Salmonella*, *Brucella*, or even

*Burkholderia pseudomallei*.<sup>9,12,13</sup> Among the fungal diseases, necrotizing granulomas are often associated with histoplasmosis; coccidioidomycosis can cause both necrotizing and pyogenic granulomas, and blastomycosis primarily causes pyogranulomas. Radionuclide studies with technetium 99m and other bone-seeking chemicals may be used to detect clinically occult lesions. In the absence of evident fungal infection at sites other than the musculoskeletal system, biopsy of the osseous lesion is required.

## Joint disease

With the exception of some infections, such as those due to *B. dermatitidis* and *Candida*, in which the onset may resemble an acute bacterial septic arthritis, most cases of fungal arthritis have an indolent presentation. In the absence of evident systemic infection, the clinician's attention is focused on the presence of a monoarticular or pauciarticular arthritis. Although large weight-bearing joints, such as the knees, are most commonly involved, virtually every joint in the body can be the focus of fungal infection. Thus, the initial list of differential diagnoses may be quite broad. On clinical examination, the usual findings of arthritis may be present with decreased range of motion, tenderness, and swelling. Erythema may be present in those cases with a more acute presentation. Evidence of joint effusion is present, but in some cases of chronic infection with organisms such as *C. immitis*, joint swelling may be due to synovial proliferation rather than the accumulation of fluid.

Plain radiographic findings may be similar to those in tuberculosis, metastatic neoplasm, rheumatoid arthritis, sarcoidosis, pigmented villonodular synovitis, or Langerhans cell histiocytosis. Joint effusion is commonly seen, but the presence of other abnormalities depends to a great extent on the chronicity of the infection, its specific cause, and host factors, including the presence of underlying joint disease. These more variable findings include adjacent osteoporosis, erosion of juxtaarticular cortex, and frank adjacent osteomyelitis. Magnetic resonance imaging has greater sensitivity and resolution than conventional radiographic techniques and may provide a more comprehensive image of the integrity of the joint and reveal the presence of otherwise unapparent paraarticular osteomyelitis,<sup>14,15</sup> but its role in the diagnosis and management of patients with fungal arthritis has not been critically evaluated. Radionuclide techniques may confirm clinical evidence of joint inflammation.

The synovial fluid protein concentration is usually greater than 3 g/dl, and the glucose concentration may be low (Table 25-3). Although infections due to *Candida* species and *B. dermatitidis* often are seen with frankly purulent synovial fluid with neutrophil predominance, the intraarticular inflammatory response to other fungi tends to be less acute and less intense. This is reflected in lower cell counts and a variable predominance of either polymorphonuclear leukocytes (PMNs) or lymphocytes. Direct examination of synovial fluid with potassium hydroxide treatment or the Gram stain usually fails to allow visualization of the organism. Cytologic preparations, however, may be useful in the diagnosis of infections due to *Cryptococcus neoformans*, *B. dermatitidis*, and, to a lesser degree, *C. immitis*.

The diagnosis may require synovial biopsy. Histopathologic examination reveals variable and sometimes non-specific

findings and, in some infections, such as those due to *Sporothrix schenckii*, the organisms may be few and difficult to detect. When a granulomatous reaction is found in the absence of visualization of any organisms, the differential diagnosis includes not only fungal infection but also mycobacterial infection, rheumatoid arthritis, syphilis, sarcoidosis, brucellosis, pigmented villonodular synovitis, Crohn's disease, foreign body reaction, gout, pseudogout, oxalosis, and protothecosis. In addition to culture of synovial tissue, blood cultures, bone marrow examination and culture, antibody tests (e.g., for serum coccidioidal or histoplasma antibody) or tests for the detection of fungal antigen in body fluids (e.g., serum cryptococcal antigen, urine histoplasma antigen) may be of value, depending on the clinical setting and the suspected pathogen.

Acute self-limited arthritis or periartthritis in association with acute non-disseminated coccidioidomycosis has been called "desert rheumatism." It may be seen in association with erythema nodosum or erythema multiforme and hilar lymphadenopathy and thus resemble sarcoidosis. This process is thought to be the result of immunologic phenomena, probably immune complex deposition. A similar phenomenon occurs in acute histoplasmosis, as well as acute blastomycosis.

## Tenosynovitis

Fungal tenosynovitis may be the result of hematogenous dissemination or of direct inoculation and may occur in the presence of joint space infection or in association with paraarticular osteomyelitis. Tenosynovitis is most often due to candidal and non-candidal yeasts, such as *C. neoformans*, as well as *S. schenckii* and *C. immitis*.

## Causative fungi

### *Blastomyces dermatitidis*

Although *B. dermatitidis* is not generally considered an opportunistic pathogen, many patients with progressive or disseminated infection have potentially predisposing conditions such as diabetes, alcoholism, renal failure, and malignancy (see Table 25-2).<sup>16-18</sup> The clinical presentation and therapeutic response of those with underlying disease are apparently similar to those who are immune competent. Rapidly progressive and unusually severe disease may occur in patients with profoundly impaired immunity, such as transplant recipients and those with AIDS.<sup>18</sup> The low rate of infection in endemic areas (0.5 to 4 cases per 10<sup>6</sup> population) may, in part, explain the infrequency with which this disease is seen in immunologically impaired hosts.

Hematogenous dissemination follows pulmonary infection and those patients with particularly severe pulmonary disease or miliary involvement or who are immune compromised are at the greatest risk for dissemination (see Table 25-1).<sup>16-19</sup> Nonetheless, in apparently normal hosts with self-limited pulmonary disease, skeletal infection can develop.<sup>20,21</sup> Most patients with skeletal infection due to blastomycosis also present with skin and pulmonary infection.<sup>22</sup> Endogenous reactivation of skeletal disease, following initial immunologic or therapeutic control, can occur late in the course in patients with chronic pulmonary disease; the risk of reactivation appears greatest in



**Table 25-3** Clinical and laboratory data helpful in the diagnosis of fungal joint infection

Organism	Serologic tests and antigen detection	Synovial fluid WBC count	Synovial glucose	Synovial fluid examination	Cultures
<i>Candida</i> species	Beta glucan assay	Frankly purulent, <100,000/mm <sup>3</sup> polymorphonuclear	Variable, low to normal	20% positive	Blood and/or synovial fluid, >95%
<i>Coccidioides immitis</i>	Complement fixation, immunodiffusion	<50,000/mm <sup>3</sup> , mononuclear cells	Low	Rarely positive	Synovial fluid >95%
<i>Blastomyces dermatitidis</i>	Low sensitivity, low specificity	Frankly purulent, <100,000/mm <sup>3</sup> polymorphonuclear	Variable, low to normal	By cytologic preparation, 88% positive	Synovial fluid 50%
<i>Sporothrix schenckii</i>	Not available	2,000–60,000/mm <sup>3</sup> lymphocytes and polymorphonuclear	Variable, low to normal	Rarely positive	Synovial tissue >synovial fluid
<i>Histoplasma capsulatum</i>	Complement fixation, immunodiffusion; antigen test			Not helpful	Blood and/or synovial fluid 20%–25%
<i>Cryptococcus neoformans</i>	Cryptococcal antigen diagnostic	200–5,000/mm <sup>3</sup> , no particular cellular predominance	Variable, usually normal	India ink	Blood and/or synovial fluid >80%
<i>Paracoccidioides brasiliensis</i>	Serum antibody			Occasionally helpful	Usually positive, slow growth (>4 wk)

Modified from Kemper CA, Deresinski SC: Fungal arthritis. In Maddison PJ, Isenberg DA, Woo P, Glass DN (eds): Oxford Textbook of Rheumatology. Oxford University Press, Oxford, United Kingdom, 1993.

the first 2–3 years after the primary pulmonary infection.<sup>19</sup> Patients with AIDS have been described who had potential exposures occurring years before diagnosis of their infection, suggesting late reactivation.<sup>16</sup> Cutaneous inoculation, usually as a result of accidental exposure in the laboratory, during post-mortem examination<sup>23,24</sup> or as a result of trauma,<sup>25</sup> is rare.

Myalgias and arthralgias are common during the acute pulmonary infection, and a reactive arthritis similar to that seen in coccidioidomycosis, often preceding the recognition of pulmonary blastomycosis by several weeks, has been reported.

#### Bone disease

Osseous sites, along with the skin, are among the most common loci of extrapulmonary blastomycosis, with the former being involved in 7–48% of cases of disseminated infection.<sup>26–32</sup> Some authors have noted an increased likelihood of bone involvement and less frequent central nervous system involvement in patients infected in Africa.<sup>33</sup>

Although any bone can be involved, the most common sites of osseous involvement include the lumbar and thoracic vertebrae; long bones (particularly the tibia); ribs; small bones of the hands, wrists, feet, and ankles; pelvis; facial bones; and skull.<sup>22,32,34,35</sup> Gehweiler and colleagues reviewed the location of 89 osseous lesions in 45 cases, finding the ribs (15% of sites), tibia (14%), and vertebrae (11%) to be the most common sites

of involvement.<sup>34</sup> McDonald et al reported that skeletal disease was the most common extrapulmonary manifestation of infection in their patients, occurring in 17 of 72 cases (with a total of 20 osseous lesions).<sup>36</sup> Soft tissue swelling and deep tissue abscesses contiguous to sites of osteomyelitis may be seen, and sinus tracts may develop. Dissection may lead to sinus tract formation distant from the site of bone involvement. Neuritis and spinal cord compression can occur as a result of extension of infection from sites of sacral or vertebral disease.<sup>20</sup>

Radiographs reveal osseous lesions that are primarily lytic, with well-circumscribed sclerosis, little or no periosteal reaction, and no formation of sequestra. In long bones, a “saucer”-shaped erosion of the cortex may be seen. Occasionally, a moth-eaten pattern of osteolysis may be seen, presumably associated with more rapid bony destruction. Differentiation of blastomycotic bone disease from tuberculosis, malignant disease or other fungal disease, on the basis of the radiographic presentation, is difficult. Vertebral lesions, resembling those of tuberculosis, slowly destroy the anterior vertebral body and disk space. In contrast to tuberculosis, which seldom involves ribs or a more distant vertebral body, extension of blastomycosis from a paraspinal abscess to ribs or along the anterior longitudinal ligament to a distant vertebral body is common.

The identification of the organism is primarily based on its visualization in tissues and culture. Extensive necrosis and

suppuration are found on histopathologic examination of infected bone, but granulomata may also be seen. Fine-needle aspiration and cytology may be diagnostic.<sup>37</sup> With the possible exception of epidemiologic investigations during outbreaks, skin testing is not of diagnostic value, and a high proportion of patients with disseminated or progressive disease are anergic. Complement fixation serologic tests have also not proven to be of value. Enzyme immunoassay and immunodiffusion studies for detecting serum antibody to *B. dermatitidis*, the exoantigen test for identifying the mycelial form in culture, and the fluorescent antibody technique for detecting and identifying yeast-form cells in culture or tissue have been useful.<sup>18</sup>

### Joint disease

Joint infection is a less frequent manifestation of extrapulmonary blastomycosis than is osteomyelitis, occurring in 2.5–8% of patients with systemic disease.<sup>29,32,36,37</sup> Joint infection occurs as a result of direct hematogenous spread or extension of juxtaarticular osteomyelitis. Rarely, it results from direct inoculation in the setting of trauma. A case of chronic blastomycotic arthritis with dissemination to skull after knee arthroscopy has been reported.<sup>38</sup>

Of all the fungal arthritides, blastomycotic arthritis is among the most likely to be confused with acute bacterial infection. It is characteristically monoarticular (95% of cases) and most commonly involves the knee, followed by the ankle, elbow, and wrist. Joint pain is often acute in onset, and patients often appear toxic. Active pulmonary disease is present in 89–100% of patients with joint involvement, and 72–92% have evidence of additional dissemination to cutaneous or subcutaneous sites.<sup>39,40</sup> Synovial fluid is usually cloudy or frankly purulent with white blood cell counts, which may exceed 100,000/mm<sup>3</sup> with a predominance of PMNs (see Table 25-3).<sup>39-41</sup> The concentration of protein in the synovial fluid usually exceeds 3.0 g/dl, whereas the glucose concentration is normal or low. Less than one-third of patients have radiographic evidence of juxtaarticular osteomyelitis.

In contrast to the infrequency with which the organism can be visualized in synovial fluid in most other fungal arthritides, *B. dermatitidis* can be seen microscopically in most cases.<sup>37,39-41</sup> Bayer and colleagues described nine patients who underwent joint fluid examination, all but one of whom had characteristic organisms by direct microscopy and three-fourths of whom had positive cultures.<sup>39</sup> The organism may also be recovered in culture or visualized on histopathologic examination from synovial biopsy specimens. Histopathologic examination of infected synovium may reveal prominent PMN infiltration and microabscesses as granulomas or both.

### Management

Limited data are available specific to therapy of osteoarticular infections<sup>22</sup> which must largely be extrapolated from series dealing with the treatment of blastomycosis considered more broadly. Amphotericin B deoxycholate therapy is associated with an excellent rate of response and a relatively low relapse rate.<sup>42,43</sup> Limited published data concerning the use of lipid formulations of amphotericin B are available, but at least one has been demonstrated to be effective in a murine model of infection.<sup>44</sup> Ketoconazole therapy has been associated with efficacy rates as high as 85%, but also with high relapse rates,<sup>43,45,46</sup> but its toxicity in high dose is problematic. Ketoconazole has

been replaced in the armamentarium by itraconazole, which has been associated with success rates as high as 95%.<sup>47,48</sup> The initial experience with low doses of fluconazole was disappointing,<sup>49</sup> but better results were obtained at doses of 400–800 mg daily.<sup>50</sup> Both voriconazole and posaconazole are active in vitro against *B. dermatitidis*. Clinical experience with each of these is extremely limited, however, although successful salvage therapy with voriconazole has been reported.<sup>51</sup>

Treatment is required for all episodes of disseminated extrapulmonary disease, including osseous infection. Amphotericin B remains the initial drug of choice for some patients, particularly those who are critically ill, have evidence of progressive disease, or who are severely immunocompromised. In these cases, treatment may be initiated with a lipid formulation of amphotericin B at a dosage of 3–5 mg/kg daily, or with conventional amphotericin B at a dosage of 0.7–1 mg/kg daily. Once the clinical status of the patient has sufficiently improved, itraconazole 200 mg three times daily for 3 days and then 200 mg twice daily may be substituted. In patients whose disease is not immediately life threatening, initial polyene therapy is not necessary. The serum concentration of itraconazole should be measured after at least 2 weeks of therapy, especially if the tablet formulation is prescribed, in order to assure adequate drug exposure. Treatment should generally be continued for at least 6–12 months. In addition to chemotherapy, surgical debridement of affected soft tissue, bone and/or synovium is often a critical part of management.

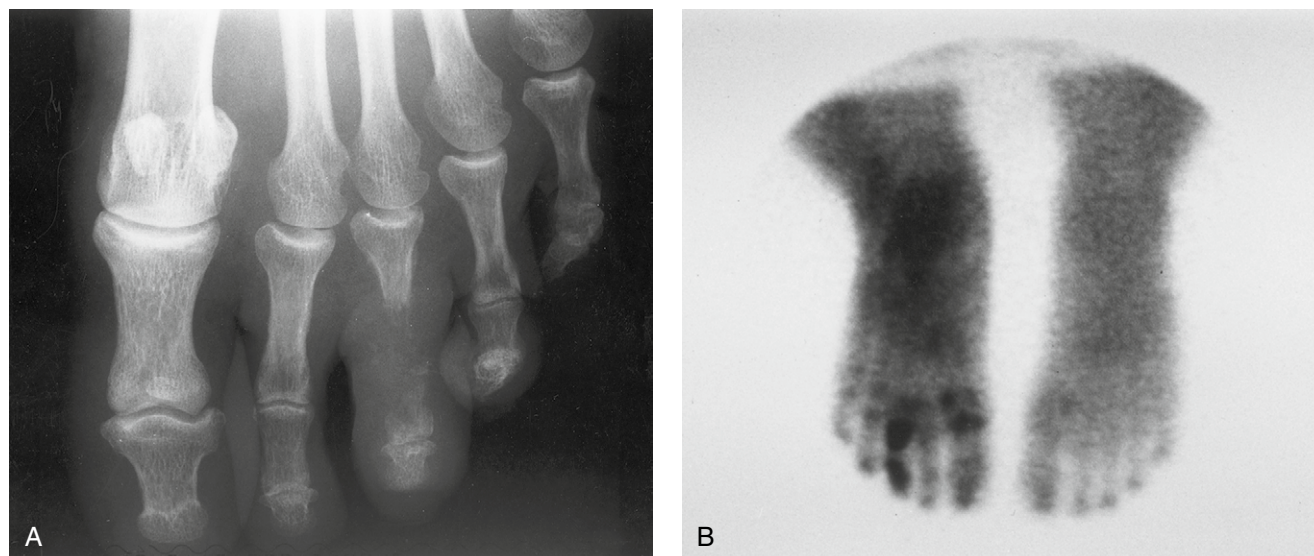
### Candida species

Candidal bone or joint disease most frequently occurs in neonates and in immunosuppressed patients with a prior episode of fungemia or in the setting of ongoing widespread dissemination. Up to 15% of patients undergoing bone marrow transplantation or of those who have prolonged episodes of neutropenia may develop candidemia, and are at particular risk for candidal bone and joint disease. People who have received prolonged immunosuppressive therapies, systemic corticosteroids, hyperalimentation, and broad-spectrum antimicrobials are also at risk for fungemia and its later sequelae.<sup>52</sup> In contrast, patients with AIDS, who primarily have deficits in natural killer cell and T lymphocyte function but not necessarily abnormalities of granulocyte and macrophage function, are not at high risk for disseminated candidiasis.

### Bone disease

*Candida* osteomyelitis, like arthritis, is rare and usually occurs via hematogenous seeding, but it may also result from inoculation during surgery or trauma (see Table 25-2) or from contiguous infected foot ulcers in diabetic patients (Fig. 25-3). Candidal osteomyelitis is most commonly due to *C. albicans* but several cases due to *C. parapsilosis*, *C. tropicalis*, and *C. glabrata* have been described. The development of *Candida* osteomyelitis in a patient, particularly a child, without apparent predisposing factors should lead to a search for evidence of PMN dysfunction, such as myeloperoxidase deficiency and chronic granulomatous disease.<sup>53</sup>

A review published in 1987 found a total of only 53 cases of candidal osteomyelitis reported in the literature.<sup>54</sup> Seventy percent were adults; six represented instances of contiguous osteomyelitis – four after median sternotomy and one each



**Figure 25-3** Roentgenograms (A) and technetium pyrophosphate nucleotide scan (B) in a diabetic patient with osteomyelitis and tenosynovitis due to *Candida albicans*. The patient presented with a diabetic foot ulcer of the third toe, which failed to heal despite prolonged antibiotic therapy. Plain films showed destruction of the proximal and middle phalanges (A). The bone scan showed corresponding activity, with diffuse labeling over the tarsal bone most consistent with increased perfusion (B). On resection, the bone was grossly involved, as was the extensor hallucis longus tendon and tendon sheath (Reproduced with permission from Kemper CA, Deresinski SC. Fungal disease of bone and joint. In: MacKenzie DWR, Odds FC, Kibbler CC (eds) Principles and Practice of Clinical Mycology. John Wiley, Chichester, Sussex, 1996).

after laminectomy and oral surgery. Thirty-one of the adult cases were hematogenous in origin, but the onset of symptoms of osteomyelitis was delayed for as long as 15 months after an episode of candidemia. Patients most commonly had infection of either a single long bone or two contiguous vertebral bodies, but infection was polyarthritic in six patients. Osteomyelitis developed in eight patients despite the prior administration of amphotericin B given because of candidemia. The most common presenting complaint of candidal osteomyelitis is local pain, but soft tissue swelling, contiguous abscess, and adjacent arthritis also occur.<sup>54,55</sup> Fever is frequently absent, and the white blood count is frequently normal. Despite earlier evidence of fungemia, blood cultures for fungus may be negative at the time of presentation with osteomyelitis.

Ferra et al described the incidence of fungemia (3.9%) and osteomyelitis (0.66%) in 305 patients who underwent bone marrow transplantation.<sup>55</sup> Despite initial treatment for an episode of fungemia, two of 13 cases developed osteomyelitis at 5 and 14 months after treatment. The authors concluded that fungemia, at least in bone marrow transplant patients, is a significant risk factor for osteomyelitis and that such patients should receive antifungal aggressive therapy and immediate removal of indwelling central venous catheter devices.

#### Joint disease

A wide variety of candidal species have been reported to cause joint infection, including *C. albicans*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, and *C. zeylanoides*. Candida arthritis is most often the result of hematogenous dissemination, frequently from infected indwelling intravenous catheters or as a consequence of illicit intravenous drug use.<sup>56</sup> Despite severe abnormalities in immune function, the presence of HIV disease does not seem to predispose to disseminated candidiasis or to candidal arthritis; only isolated

cases have been reported, most of whom were injection drug users.<sup>57,58</sup> Joints involved by rheumatoid arthritis seem to be at increased risk of infection by *Candida*.<sup>59</sup> Some infections result from direct inoculation of the organism into the joint during aspiration or injection of corticosteroids<sup>59</sup> or as the result of trauma or surgery, such as arthrotomy.<sup>60</sup>

The large joints are most commonly affected. The onset of disease is subacute in approximately one-third of patients, and some presentations are remarkably indolent. An acute onset of disease seen in the other two-thirds of patients distinguishes this cause of fungal arthritis from many of the others discussed here.<sup>61</sup> The frequently very high synovial fluid white blood cell counts (15,000–100,000/mm<sup>3</sup>), with a predominance of PMNs, also distinguishes this infection from most other fungal arthritides (see Table 25-3). The synovial fluid glucose may be low, whereas the protein concentration is elevated. Histologic examination of synovium obtained beyond the very first days of infection reveals a mononuclear cell infiltration, but granulomas are usually absent. Direct examination of synovial fluid by Gram stain or other methods results in visualization of the organism in only 20% of cases. On the other hand, culture of synovial fluid or synovium yields the organism in a high proportion of cases. In some patients, blood cultures may also be productive of the pathogen.

Almost one-fifth of cases of nosocomial septic arthritis in neonates are caused by *Candida* species.<sup>62,63</sup> Neonatal *Candida* arthritis is usually just one part of a systemic infection, with involvement of multiple sites, and is frequently associated with broad-spectrum antibiotic therapy and parenterally administered nutrition, as well as with prematurity, abdominal surgery, malnutrition, and immunosuppressive disease or therapy.<sup>64,65</sup> As a result of the systemic nature of the infection in neonates, the organism may frequently be recovered from blood, urine, and cerebrospinal fluid, as well as from joint



fluid. There is usually little difficulty in recovering the organisms from the latter site; joint aspirates yielded the offending pathogen in every case in the largest series reported.<sup>62</sup> Polyarticular infection was seen in one-third of cases; at least one knee was involved in 71%.

As seen in adults with *Candida* arthritis, the synovial fluid white blood cell count in neonates is as high as 100,000/mm<sup>3</sup> and PMNs predominate. Radiographic evidence of adjacent osteomyelitis has been seen in two-thirds of patients and in almost 90% of joints.<sup>62</sup> This observation suggests that, at least in those cases, the joint was infected as the result of rupture into the articular cavity from infection that had originated at the metaphysis.<sup>66</sup> Subluxation of the femoral head may be seen in some cases of hip joint infection. Gross examination of the synovial membrane revealed it to be hyperemic and purulent and the cartilage eroded. Major orthopedic sequelae were seen in only one-tenth of survivors, but the mortality rate was 14%.

Although fungi may infect prosthetic joints, they do so rarely.<sup>67,68</sup> These prosthetic infections most likely result from the inoculation of skin microflora at the time of implantation. Patients often present late after their initial arthroplasty. In one review they presented 5–36 months later with low-grade infections manifested by pain and decreased range of motion with periarticular swelling. Loosening and osteolysis adjacent to the prosthesis, findings potentially indicative of infection, are often seen, and sinus tracts may be present. Radionuclide techniques are often not useful, because technetium pyrophosphate and gallium nitrate scans are routinely positive in the presence of a loosened prosthesis, regardless of the presence of infection. The value of indium 111 white blood cell scanning is unknown. Consistent with the more indolent presentation, synovial fluid white blood cell counts are lower (4000–15,000/mm<sup>3</sup>), with a predominance of PMNs.

### Management

In patients in whom osteoarticular infection is part of an ongoing disseminated infection, treatment strategies are dictated by the latter factor, and most clinicians would choose an amphotericin B preparation or an echinocandin as initial therapy. The largest reported experience is with amphotericin B, although caspofungin has been effective in a small number of patients with osteoarticular candidiasis.<sup>69,70</sup> In less severe cases, therapy may be initiated with a triazole, usually fluconazole, which may also be used for completion of therapy in patients initially treated with amphotericin or an echinocandin, if in vitro testing demonstrates susceptibility. Effective debridement is a critical part of therapy in many cases and the placement of bone cement containing amphotericin B has been utilized, but the benefit of this treatment is uncertain.<sup>71</sup> The appropriate total duration of therapy is also uncertain, but is generally recommended to be several months to as long as a year.

While repeated joint aspirations have been used in the past, it is likely that debridement and irrigation of infected joints, preferably with use of an arthroscope, are preferable. Infected prosthetic devices appear to invariably require removal. Delayed reimplantation has been successfully accomplished.<sup>72</sup> Systemic administration of amphotericin B and fluconazole has been demonstrated to lead to acceptable synovial fluid concentrations of the antifungal agent.<sup>73,74</sup> There is no indication for intraarticular administration of antifungals in these patients. The strategy for antifungal therapy is the same as

with bone infection, although shorter durations of therapy may be acceptable.

### *Coccidioides* spp.

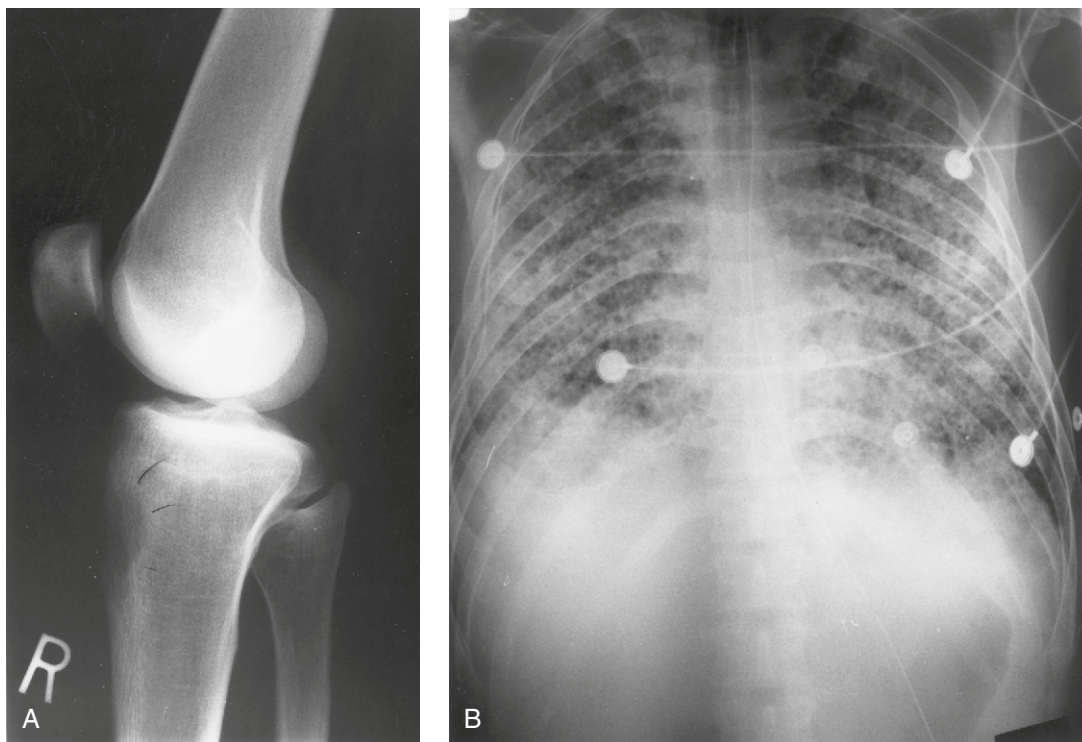
Anthropologic data support the likely occurrence of skeletal disease due to *C. spp.* in endemic areas for centuries.<sup>75</sup> Although extrapulmonary dissemination of coccidioidomycosis occurs in approximately 0.5% of infected individuals, infection of the skeletal system is one of the most frequent manifestations of dissemination, occurring in 10–42% of cases (see Table 25-1).<sup>76</sup> Approximately half of those with disseminated disease are immunocompromised by diabetes, renal failure, immunosuppressive therapeutics or corticosteroids (see Table 25-2).<sup>76-78</sup> Patients with HIV infection are at increased risk for more frequent and severe coccidioidomycosis, although there is some controversy as to whether disease occurs as a result of reactivation of infection or as a result of recent exposure.<sup>79,80</sup> Widespread dissemination, with cutaneous lesions, meningitis, and bone disease (Fig. 25-4), can occur in patients with HIV disease, but in many of these patients rapidly progressive and often fatal pulmonary disease manifested by diffuse interstitial and nodular infiltrates may first develop.<sup>81,82</sup>

### Bone disease

Bone involvement occurs almost exclusively as a result of hematogenous dissemination. Extension of pulmonary, cutaneous, or oral or nasal mucosal disease to bone is rare. Two-fifths of cases are polyostotic: 20% involve two bones, 10% involve three bones, and 1% involve as many as eight bones (Fig. 25-5). Any bone may be infected but the most common sites of involvement include the lumbar and thoracic vertebrae, followed by the tibia, skull, metacarpals, metatarsals, femur, and ribs.<sup>75,77,81</sup> Involvement of the long bones, ribs, and small bones of the hand may be curiously symmetric.<sup>83</sup> Lesions most commonly occur in the middle of flat bones or in the metaphysis of long bones, with a special predilection for bony prominences, such as the tibial tuberosity (see Fig. 25-4A), the malleoli, and the styloid processes. In the bones of the hands and feet, the diaphysis is commonly involved (Fig. 25-6). Multiple vertebral lesions are common and contiguous vertebrae are often affected, but the disk is apparently spared. In addition, the vertebral pedicle, transverse processes, and spinous process may each be separately involved. Contiguous rib involvement may occur in thoracic vertebral disease. In contrast, only one-tenth of cases of vertebral tuberculosis involve more than one vertebra, the vertebral body is primarily affected, the disk is often spared, and spread to adjacent ribs is uncommon.

The clinical presentation is varied and depends on the site and chronicity of bone involvement. Early bone infection may be heralded by acute pain accompanied by focal erythema, swelling, and palpable tenderness. At the other end of the spectrum, many chronic lesions are non-tender, with “cold” abscesses and chronically draining material (see Fig. 25-2). Soft tissue abscesses and draining sinus tracts, often connecting to foci of osteomyelitis, occur in one-tenth of cases.

On radiographic studies, skull and vertebral lesions are lytic, with distinct margins and little or no evidence of new bone formation or sclerosis; many lesions appear “multiloculated.” However, lesions of the small bones of the hands and



**Figure 25-4** (A) Well-circumscribed lytic bone lesion in the proximal tibia of a 32-year-old man with AIDS due to *C. immitis*. The patient complained of severe shortness of breath and focal pain of the knee. Examination of the knee revealed no evidence of erythema or dolor, but the tibial tuberosity was exquisitely tender. (B) Severe miliary pulmonary disease was seen on chest radiograph. *C. immitis* has a predilection for bone prominences, such as the tibial tuberosity.

feet can be less distinct with an irregular moth-eaten appearance. Periosteal elevation is uncommon.<sup>83</sup> Sclerosis and cortical thickening are generally “late” findings in response to chronic destruction of bone (Fig. 25-7). With healing, the affected area generally undergoes sclerosis, although a normal radiographic appearance, particularly in children, may result years later.

In patients with isolated osseous coccidioidal infection, systemic symptoms such as fever, sweats, and weight loss are generally absent or mild. Anemia and leukocytosis may be present, and many patients manifest an erythrocyte sedimentation rate of 100 or greater. Hypercalcemia may also occur. The complement fixation test is useful in establishing the diagnosis and is a marker of therapeutic response. Technetium pyrophosphate bone scans are useful in the diagnosis of this disease; they are more sensitive than conventional radiographs and may direct the clinicians to occult sites of bone (and joint) involvement (see Fig. 25-5D).<sup>84</sup> Magnetic resonance imaging may also prove to be useful.

#### Joint disease

Joint space infection occurs in up to 25–30% of individuals with disseminated coccidioidomycosis (see Table 25-1). Although organisms may reach the joint space and synovial tissues directly by way of the bloodstream, septic arthritis often develops as a result of extension of infection from an adjacent site of osteomyelitis (see Fig. 25-7). It is often difficult to determine the initial site of infection in these cases. As is the case with bone infection, joint infection can remain occult for months. At the time of detection of disseminated

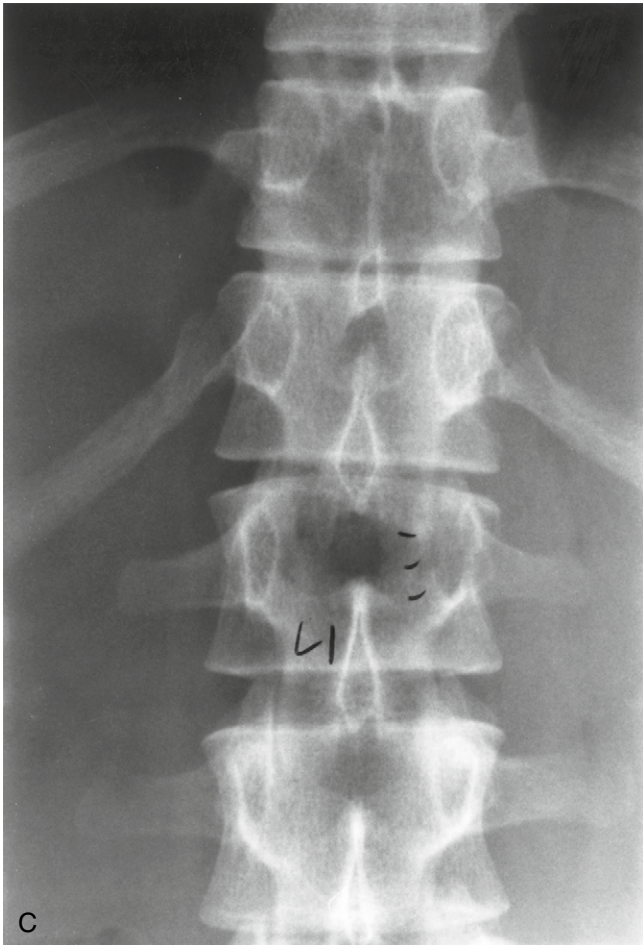
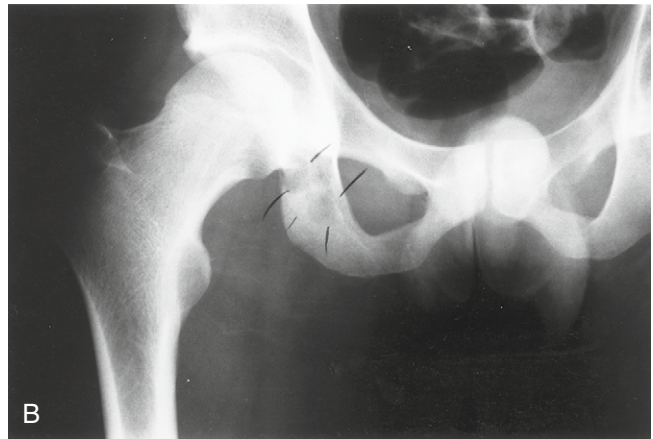
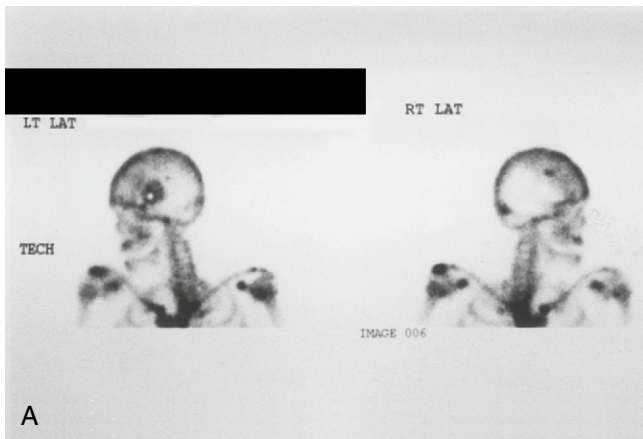
infection, up to one-fourth of joint infections may not be clinically apparent.<sup>85</sup> Bone and joint infection should therefore be avidly sought for in any patient with suspected or apparent disseminated infection.

In a review of 42 patients with joint infection, only a single joint was infected in greater than 90%.<sup>75</sup> The knee was involved in 32 (76%) of these (one patient had bilateral knee infection). The remaining patients had involvement of the ankle (10%), elbow (5%), wrist (2%), hip (2%), and interphalangeal joints (2%). Although the large weight-bearing joints are most commonly involved in adults, the small joints of the hands and feet may be more commonly affected in children (see Fig. 25-6).<sup>86,87</sup>

Some, but not all, patients are seen with an acutely inflamed joint. More often, the patient has complaints of chronic joint pain and stiffening. With few clinical signs of infection, except evidence of limited joint mobility, the indolent nature of the infection often leads to misdiagnosis. Progressive infection with effusion and synovial proliferation gradually results in severe destruction and loss of joint integrity and function. Occasionally, chronic arthrocutaneous fistulas develop with drainage of synovial fluid. Baker’s cysts may occur as a consequence of knee involvement.

Radiographic examination during the initial phase of the infection may be unremarkable, but subsequent examinations may reveal joint space narrowing in two-thirds, evidence of intraarticular effusion (in ankles and knees) in three-quarters, and periarticular periostitis in half. Erosion of articular cortex, often in areas of adjacent osteoporosis, is more common as the





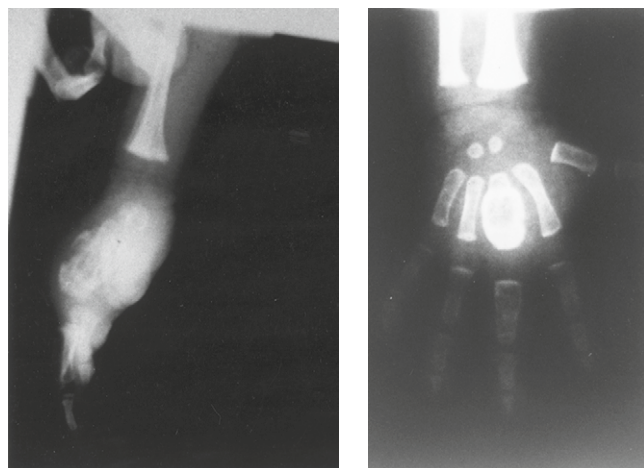
**Figure 25-5** *Coccidioides immitis* infection of the skull (A), lumbar vertebrae (B), and sacrum (C) in a 20-year-old Filipino college student living in California. Infection has resulted in an irregular moth-eaten appearance of osteolysis of multiple bones with no evidence of sclerosis or new bone formation. Approximately two-thirds of patients with skeletal disease due to *C. immitis* have two or more sites of involvement. Many may be occult and require technetium pyrophosphate radionuclide scanning for detection, as in this young man (D). The technetium scan revealed multiple foci of intense uptake in the skull that were not appreciated on plain films (from Kemper CA, Deresinski SC. Fungal disease of bone and joint. In: MacKenzie DWR, Odds FC, Kibbler CC (eds) *Principles and Practice of Clinical Mycology*. John Wiley, Chichester, Sussex, 1996).

infection progresses.<sup>88</sup> Technetium radioisotope scans usually localize to affected joints.<sup>84</sup>

The synovial fluid is inflammatory, with total white blood cell counts ranging from 5000/mm<sup>3</sup> to as high as 50,000/mm<sup>3</sup> (see Table 25-3). Synovial fluid may reveal either a predominance of PMNs or mononuclear cells. Protein is greater than 3 g/dl, glucose is low, and mucin clot is poor. Culture of synovial fluid yields the organism in one-half of cases, usually within 3–6 days, but a greater yield is seen with culture and histologic examination of synovial tissue. If coccidioidomycosis infection is suspected, the microbiology laboratory must

be notified because of the significant biohazard represented by this organism in culture. The affected proliferative synovium (see Fig. 25-1A, B), which often invades cartilage and articular surfaces, exhibits granulomatous villonodular inflammatory changes, with the characteristic endospore-forming spherules visible on microscopic examination. Serum complement fixing antibody to coccidioidin is almost universally present, with the height of the titer generally reflecting the extent of dissemination, as in other forms of infection with this organism.<sup>76</sup> Delayed dermal hypersensitivity to coccidioidin may be absent.





**Figure 25-6** Osteomyelitis due to *Coccidioides immitis* of the digit in a 5-month-old child; the infection caused osteolysis with a narrow margin of sclerosis in the diaphysis. This was the sole site of infection in this child.



**Figure 25-7** Long-standing osteomyelitis of the distal femur and proximal tibia, with extension into the knee joint, resulting in destruction of the joint and adjacent bones (courtesy of David A. Stevens MD).

Transient arthralgias and an aseptic inflammatory arthritis (“desert rheumatism”) occur in approximately 3–5% of those with acute primary coccidioidomycosis. This aseptic inflammatory process is responsive to treatment with non-steroidal anti-inflammatory agents.

### Management

The choice of antifungal therapy of osteoarticular infection follows the same principles as outlined in the guidelines of the Infectious Disease Society of America.<sup>89</sup> When such infections are part of ongoing disseminated disease, an amphotericin B preparation is generally initially administered, with deescalation to orally administered triazole therapy once the disease is stabilized. Clinical trial data suggest that itraconazole is superior to fluconazole in the treatment of patients with osteoarticular infection.<sup>90</sup> In a randomized, controlled clinical trial, 191 patients with non-meningeal coccidioidomycosis, 50 of whom had skeletal involvement, received either fluconazole

400 mg daily or itraconazole 200 mg twice daily for up to 12 months. The primary endpoint was success defined as clinical improvement of at least 50% after 8 months of therapy. At that point, success was achieved in 50% and 63% of fluconazole and itraconazole recipients, respectively ( $P=0.08$ ), while at 12 months, success was achieved in 57% and 72%, respectively ( $P=0.05$ ). Among the patients with osteoarticular infection, success at 12 months was achieved in 37% of fluconazole recipients and 69% of those given itraconazole ( $P=0.05$ ). Thus, evidence favors the use of itraconazole over fluconazole in the treatment of osteoarticular coccidioidomycosis.

There is limited published information regarding the use of voriconazole in the treatment of coccidioidomycosis. Posaconazole appears to have promise. Therapy with posaconazole elicited favorable responses in 5/6 patients with skeletal coccidioidomycosis, although one patient subsequently relapsed.<sup>91</sup> Separately, clinical improvement was achieved in 4/5 patients with osteoarticular infection who had failed or were intolerant to previous antifungal therapy.<sup>92</sup>

Surgical intervention is key to the management of the majority of cases of osteoarticular coccidioidomycosis. Bone lesions may require debridement and sinus tracts, if present, should be excised. Synovectomy is often effective in the management of coccidioidal arthritis. Of nine patients treated with combined surgical debridement and amphotericin B, seven were disease free 4–12 years later but in two patients, disease recurred.<sup>93</sup> Despite medical and surgical treatment, the infection often remains progressive and disabling.

Patients with AIDS and disseminated coccidioidomycosis, including skeletal involvement, should probably receive a lipid formulation or conventional amphotericin B, followed by lifelong suppressive therapy with itraconazole or fluconazole. Despite aggressive therapy, progression of disease and relapses are frequent.

## *Cryptococcus neoformans*

*Cryptococcus neoformans* is worldwide in distribution, and skin test surveys suggest that subclinical infection is common in normal hosts. Although some patients with cryptococcosis lack obvious abnormalities of immune function,<sup>94,95</sup> most have some impairment of immunity, such as renal failure, diabetes, connective tissue disorders, alcoholism, hemoproliferative disorders, or transplant recipients. People with AIDS are particularly vulnerable to this encapsulated yeast. The portal of entry is the respiratory tract, and disseminated disease occurs as a result of hematogenous seeding. Almost any organ system can be involved, but the organism has a particular predilection for the brain and meninges.

### Bone disease

Skeletal disease due to cryptococcosis is unusual. The first reported case, a lesion of the tibia that eroded into the knee joint, was described in 1894 by Busse and Buchke. Since then a number of reviews have suggested that the incidence of bone disease in patients with cryptococcosis is 5% or less (see Table 25-1).<sup>96–99</sup> Because most patients with cryptococcal skeletal disease do not have evidence of pulmonary or meningeal disease, bone disease is believed to be the result of hematogenous spread from a focus of self-limited pulmonary or lymphatic infection in a relatively competent host. In contrast, bone involvement

in patients with AIDS and cryptococemia or meningitis is comparatively rare. Such patients may have positive cultures of bone marrow aspirates but no evidence of bone disease.

Bone disease is often indolent and may remain clinically silent for long periods of time. In one review, the duration of symptomatic disease before diagnosis was 3 months.<sup>99</sup> Most patients present with local pain of several weeks duration, soft tissue tenderness and swelling, and an absence of systemic symptoms. Behrman and colleagues described 39 patients with bone disease, of whom only 18% had fever and none had leukocytosis.<sup>99</sup> The erythrocyte sedimentation rate may be elevated in some patients but normal in others. Most patients with cryptococcal skeletal disease present with a single isolated lesion. Ong and Prathap reviewed 16 cases, nine of which involved a single bone.<sup>97</sup> Behrman and colleagues described 59 lesions in 39 patients; 74% of these patients had only a solitary skeletal lesion, 13% had two, and 10% had a maximum of six lesions.<sup>99</sup> The most common site of involvement was a vertebral body (15% of sites) but the tibia, ribs, ileum, and femur were involved in approximately 10% each. The humerus, scapula, clavicle, sacrum, and skull were less frequently involved. Many lesions also involve contiguous bone and adjacent joints.

Radiographic studies demonstrate characteristic well-defined, discrete lytic lesions without marginal sclerosis or periosteal change.<sup>97-99</sup> Certain chronic lesions can have a sclerotic appearance. Contiguous vertebral bodies may be involved, but the intervertebral disk space is usually spared.<sup>95</sup> Computed radiographic scans define the extent of bone involvement and often demonstrate a surrounding soft tissue inflammatory mass.<sup>100</sup> Technetium bone scans show increased uptake, but occasional lesions can be "cold."

The diagnosis is commonly made on the basis of cytopathologic examination and culture of aspirate or biopsy material. In one series, the diagnosis was made on the basis of aspiration alone in 20% of cases, incision and drainage in 8%, and surgery in 67%.<sup>99</sup> In those reports that provided diagnostic details, cultures were positive in about half of aspirates and most biopsy specimens. Occasionally, cultures or smears of material from a draining sinus may be diagnostic. Histopathologic examination of bone biopsy specimens may reveal gelatinous granulomatous material with occasional giant cells and extensive fibrosis. The sensitivity and prognostic value of serum cryptococcal antigen in patients with bone disease are not known.

#### Joint disease

Cryptococcal arthritis occurs less frequently than does osteomyelitis.<sup>101-103</sup> Most patients have abnormalities in cell-mediated immunity.<sup>102,104,105</sup> Joints with preexisting pathology, such as calcium pyrophosphate disease or gout, may be at increased risk of infection.<sup>106</sup> In one series of 14 patients with cryptococcal arthritis, eight (57%) had evidence of dissemination to other sites. Four of these patients had evidence of dissemination to skin, three had fungemia, and three had meningitis.<sup>102</sup> Contiguous areas of osteomyelitis were seen in one-third, supporting the belief that most cases of joint disease occur as a result of hematogenous seeding of a parasynovial site. A case of bilateral joint disease occurred in a diabetic patient with positive cultures of synovium, contiguous bone, sputum, and cerebrospinal fluid.<sup>105</sup>

The infection is often indolent but may be associated with cellulitis or significant soft tissue swelling and inflammation.<sup>107</sup> The knee is involved in approximately 60% of reported cases, followed by an equal number of cases in the sternoclavicular and acromioclavicular joints, elbow, wrist, and ankle. Approximately one-third of cases are polyarticular. Patients may have normal peripheral white blood cell counts and normal erythrocyte sedimentation rates. Roentgenograms demonstrate an erosive arthritis with areas of contiguous osteomyelitis, and CT scans may show evidence of a parasynovial inflammatory mass. Synovial fluid analyses reveal white blood cell counts of 200–20,000/mm<sup>3</sup> with a predominance of mononuclear cells.

A single case of cryptococcal bursitis, possibly as a result of accidental inoculation of the organism during needle aspiration, has been described.<sup>108</sup>

#### Management

The rarity of osteoarticular infection due to *C. neoformans* makes any definitive statement specific to the therapy of this complication difficult. If it is part of a potentially life-threatening systemic infection, treatment may be initiated with amphotericin B, often in combination with 5-flucytosine, with subsequent switch to fluconazole. In less immediately threatening forms of infection, treatment may be initiated with fluconazole. Other triazoles are active in vitro and their use may be considered in selected cases.

### *Histoplasma capsulatum*

In the normal host, acute infection with *H. capsulatum* results in an influenza-like respiratory illness in up to 5% of those infected, but most infections remain subclinical.<sup>7,109</sup> Hematogenous dissemination is believed to occur in virtually all patients during the acute primary infection, but it is usually self-limited and seldom causes significant disease. Severe progressive dissemination, due to either acute primary infection or reactivation disease, occurs most commonly in patients with impaired cellular immunity (see Table 25-2).<sup>110</sup> Disseminated histoplasmosis has emerged as an important opportunistic infection in persons with AIDS in endemic areas,<sup>111,112</sup> reportedly affecting, for example, one-third of AIDS patients in Kansas City, Missouri.<sup>113</sup> HIV-infected patients who have previously resided in or who have traveled to endemic areas are also at risk for reactivation disease.<sup>82</sup> Patients without obvious immunodeficiency in whom disseminated histoplasmosis develops should therefore be rigorously screened for HIV infection.

Erythema nodosum, erythema multiforme, arthralgias, and an immunologically mediated aseptic inflammatory arthritis, similar to that reported for coccidioidomycosis, have been described as part of primary histoplasmosis.<sup>114,115</sup> A review of previously published reports revealed that arthralgias occurred in 3–21% and that erythema nodosum and multiforme occurred in 1–42% of patients with acute histoplasmosis.<sup>3</sup> In an outbreak of acute infection in 381 symptomatic patients in Indianapolis, arthralgias occurred in 4.1% of patients and aseptic arthritis in 1.6%.<sup>114</sup> The knees, ankles, wrists, and small joints of the hands are the most common sites involved, and polyarticular involvement is common.<sup>114,115</sup> The joint involvement is rapidly additive in most patients, less commonly migratory, and involves symmetric joints in half of cases. The synovial fluid is inflammatory, with a predominance of mononuclear

cells, and sterile. These rheumatologic manifestations are typically self-limiting, and non-steroidal anti-inflammatory agents can provide symptomatic relief.

### Bone disease

In contrast to blastomycosis and coccidioidomycosis, bone and joint infection by *H. capsulatum* is rare.<sup>101,109</sup> Bone marrow involvement commonly occurs in cases of severe disseminated disease, but evidence of osteomyelitis is absent. In a series of 18 AIDS patients with histoplasmosis,<sup>112</sup> three-fourths had histologic evidence of infection on bone marrow biopsy specimens, and half had positive cultures. None had osteomyelitis or joint disease. Most of those rare cases of osteomyelitis, manifested radiographically by osteolytic lesions, have occurred in infants and children.<sup>116,117</sup> Although 7/10 children with disseminated histoplasmosis in one report had cultures of bone marrow positive for *H. capsulatum*, only one had evidence of radiolucencies in the skull, scapula, and femur.<sup>116</sup> Allen described two infants with disseminated histoplasmosis, one of whom had cortical thickening, whereas the other had a small focus of bone destruction.<sup>118</sup> Goodwin and Des Prez reported a case of vertebral disease causing spinal cord compression.<sup>109</sup>

In contrast to *H. capsulatum*, *Histoplasma capsulatum* var. *duboisii* has an apparent predilection for bone. An unusual infection seen in Africa, this agent causes multiple foci of osteolytic destruction in about half of cases. This granulomatous infection results in cortical destruction, periosteal elevation with new bone formation, and extension of infection to contiguous soft tissues with frequent fistulization and skin involvement.

### Joint disease

In addition to osteomyelitis and arthritis, *H. capsulatum* may cause tenosynovitis and carpal tunnel syndrome.<sup>119,120</sup> Infective arthritis is usually monoarticular and has been reported in both apparently immunologically normal<sup>121-123</sup> and compromised hosts.<sup>124</sup>

The diagnosis of histoplasmosis can often be made by culture of blood or bone marrow or other infected sites, such as synovial fluid, or histologic evidence of the organisms in biopsy specimens (see Table 25-3). The organism is readily cultivated on a variety of media. Blood cultures using the lysis centrifugation technique enhance recovery of the organism in patients with active dissemination.<sup>125</sup> Detection of histoplasmal antigen in serum or urine is useful in the diagnosis of disseminated histoplasmosis and provides a marker by which therapeutic success may be judged.<sup>110,126</sup> Detection of serum complement fixing antibody to the yeast phase of the organism of 1:32 or greater should be regarded as presumptive evidence of histoplasmosis. Titers of 1:8 or greater to mycelial phase antigens or the presence of “M” or “H” bands by immunodiffusion are also highly suggestive of histoplasmosis.<sup>126</sup> Histoplasmin skin testing is useful only for epidemiologic purposes.

### Management

Little can be said that is specific to the therapy of osteoarticular infection. As with other fungal infections, if it is part of an ongoing progressive and/or disseminated infection, therapy may be initiated with amphotericin B with subsequent deescalation to treatment with itraconazole. The latter may be superior

to fluconazole in the treatment of histoplasmosis. Limited information is available concerning the use of voriconazole and posaconazole, which are active against *H. capsulatum* in vitro<sup>127</sup> and in experimental infection,<sup>128</sup> with posaconazole the more active in both systems.

## *Paracoccidioides brasiliensis*

*Paracoccidioides brasiliensis* is endemic only to areas of Central and South America, where it is the most commonly diagnosed dimorphic mycosis. Although paracoccidioidomycosis is rarely encountered in the United States,<sup>129,130</sup> it should be included in the differential diagnoses of suspected fungal infection in individuals at epidemiologic risk. Paracoccidioidomycosis primarily occurs in apparently immunologically normal hosts (see Table 25-2)<sup>131-134</sup> but severe disseminated disease has been described in the immunodeficient host.<sup>132</sup> Most patients present with chronic pulmonary disease often associated with evidence of hematogenous dissemination to multiple organ systems, including painful granulomata of the skin, lymphadenopathy, and ulceration of mucous membranes. Almost any organ system can be affected in disseminated disease, including the gastrointestinal and nervous systems, bones and joints, as well as the testes, adrenal glands, and liver.

### Bone and joint disease

Osteomyelitis and joint disease due to *P. brasiliensis* are unusual. In two series describing a total of 66 cases, only one patient was described with bone marrow involvement, and none had osteomyelitis or joint disease.<sup>133,134</sup> In one report, radiographs revealed extensive moth-eaten lytic bone disease involving the femur, pelvis, calvarium, and clavicle.<sup>135</sup> A case of joint infection, with soft tissue swelling and cartilaginous destruction, has been described.<sup>137</sup> Typical budding yeast forms characteristic of *Paracoccidioides brasiliensis* were observed on direct examination of the synovial fluid, and cultures grew the organism. The diagnosis is usually made on the basis of visualization of the organism in tissues or fluids and by culture (see Table 25-3). Serologic tests have been used for diagnostic purposes with varying success, but skin tests are useful only for epidemiologic surveys.

### Management

A systematic review of the treatment of paracoccidioidomycosis<sup>136</sup> identified only a single randomized trial involving a total of 42 patients, none of whom had osteoarticular disease.<sup>137</sup> No difference in outcomes could be identified since all but one patient improved. However, amphotericin B is effective in the treatment of disseminated paracoccidioidomycosis, although itraconazole and ketoconazole are also effective in the treatment of milder disease.<sup>131,132,138</sup> The azoles, as well as the sulfonamides,<sup>131,139,140</sup> are often administered as prolonged suppressive therapy.

## *Sporothrix schenckii*

*Sporothrix schenckii* is commonly found on decaying vegetation and in soils worldwide. Infections are both sporadic and epidemic, but the prevalence of disease and the clinical presentation seem to vary in different geographic areas. In contrast to the other soil fungi discussed here, cutaneous disease



occurs secondary to inoculation as a result of trauma to the skin. The lymphocutaneous form, with the development of an ulcer at the site of cutaneous inoculation and proximal nodules in the area of lymphatic drainage, is the most common manifestation of infection. Persons at particular risk include rose cultivators and those who handle soil and sphagnum moss.<sup>141</sup> Occasionally, bites from insects, birds, and domestic or wild animals have resulted in infection. Patients in the United States seem to have a greater incidence of pulmonary and systemic disease, including skeletal disease, than has been reported in other circumstances, such as in outbreaks in South African gold miners, although these manifestations remain uncommon.<sup>142</sup> More than 80% of those with systemic disease have predisposing conditions (see Table 25-2).<sup>141,143</sup> Skeletal disease results from contiguous spread of infection from cutaneous or mucocutaneous lesion, direct inoculation, or as a result of hematogenous dissemination from a site of active or quiescent infection.

### Bone disease

Osseous sporotrichosis is an uncommon disease. In one of the original reviews of the world literature from 1898 to 1967, Wilson and colleagues identified 30 cases of systemic sporotrichosis, 24 of which involved bone or joint or both.<sup>144</sup> With an increase in the number of immunodeficient hosts, skeletal disease may, in fact, be more common. Osseous sporotrichosis is a chronic and indolent infection that may be present for months to years before diagnosis. In Gladstone and Littman's review of 22 cases of osseous sporotrichosis in 1971<sup>145</sup> the tibia and fibula were involved in eight patients (36%), the metacarpals and phalanges in six (27%), and the radius and ulna in five (23%). Nearly three-quarters had focal swelling and tenderness, and half had draining sinuses. Nearly three-quarters had evidence of concomitant arthritis. About two-thirds had distant skin lesions, but several patients had no evidence of disease elsewhere. Nineteen of 22 patients had radiographic evidence of lytic disease with little or no periosteal reaction.

Govender and colleagues reported four cases of osseous sporotrichosis involving the ulna, tibia, fibula, and ischium.<sup>146</sup> All four reported pain and had evidence of focal tenderness and minimal swelling on examination. Erythrocyte sedimentation rates varied from 47 to 58 mm/h. In contrast to Gladstone and Littman's review, however, none of the cases had evidence of cutaneous, lymphoid or pulmonary sporotrichosis, and none involved adjacent joints.<sup>145</sup> Radiographs revealed lytic lesions with evidence of periosteal reaction and new bone formation in two cases, and a thick zone of dense sclerosis in a third. The diagnosis of sporotrichosis is made on the basis of identification of the organism in culture, usually from biopsy specimens of affected bone.

### Joint infection

During the acute cutaneous or lymphocutaneous infection, approximately 2% of patients complain of arthralgias, but true joint infection develops in few. In one large outbreak of sporotrichosis involving 3300 patients, joint disease developed in only one (0.03%)<sup>147</sup> which, as described earlier, is commonly associated with infection of juxtaarticular bone (see Table 25-1).

Joint disease may possibly result from extension of cutaneous infection to contiguous synovial tissues or as a result of

direct inoculation of the organism into the joint (see Table 25-2). Although arthritis may occur in the presence of widespread infection, it is much more common as an isolated finding.<sup>143,148</sup> Bayer described 44 cases of sporotrichal joint infection, only 20% of which were associated with active systemic or pulmonary disease.<sup>143</sup> Those cases of cutaneous and pulmonary disease preceded or occurred concurrent with the joint disease. The absence of cutaneous or lymphocutaneous disease in many patients suggests a hematogenous route of infection. In those cases in which sufficient information was provided, almost 90% had underlying disease, including alcoholism, myeloproliferative disorders, malignancy, and chronic corticosteroid use.

Sporotrichal arthritis is an indolent and slowly progressive infectious process, which predominantly affects the knee and the small joints of the hand and wrist.<sup>143,149</sup> The shoulders and hips are usually spared. Monoarticular and polyarticular involvement occur with equal frequency. Calhoun and colleagues described 11 cases of systemic sporotrichosis; eight involved the skeletal system with a total of 12 joints affected, including the wrist (63%), knee (38%), ankle (25%), and elbow and phalanx (13%).<sup>150</sup> Most cases present as a slowly progressive synovitis or tenosynovitis with pain, warmth, swelling, and restricted range of motion;<sup>142,143</sup> some patients report fever.

Synovial fluid white blood cell count is reported to range from 2800 to 60,000/mm<sup>3</sup> (see Table 25-3). Both lymphocytes and PMNs may be seen. The protein concentration is high, whereas glucose is low to normal.<sup>151</sup> Radiographic abnormalities are seen in more than 90% of cases, possibly reflecting the chronicity of infection before diagnosis. Osteoporosis, osteopenia, and small lytic lesions of juxtaarticular bone are the most common findings. A joint effusion may be present, and joint space narrowing and cartilage erosion may be seen.<sup>142,143</sup>

The average time to diagnosis is approximately 2 years, varying from 2 months to 8 years in one series.<sup>143</sup> Delays in diagnosis often occur as the result of difficulty in identifying the presence of infection; many are mistakenly diagnosed as rheumatoid arthritis. Organisms are seldom visualized on smears of synovial fluid; the synovial histopathologic findings are often non-specific and may resemble that of rheumatoid or tuberculous arthritis, and there is a scarcity of organisms in tissue. Asteroid bodies, often said to be pathognomonic of sporotrichosis, may in fact be seen in other infections. Isolation of the organism in culture is the cornerstone of diagnosis; synovial tissue may be more likely to yield the organism than is synovial fluid. The organism will usually yield visible growth within 5 days. A variety of serologic tests have been used with varying efficiency, but skin tests are only useful for epidemiologic surveys.

### Management

Fluconazole appears to be ineffective in the treatment of osteoarticular sporotrichosis.<sup>152,153a</sup> Itraconazole has efficacy but prolonged treatment is required to avoid relapse.<sup>153</sup> In a non-comparative clinical trial of 30 patients with lymphocutaneous and systemic sporotrichosis, itraconazole (100–600 mg daily for 3–18 months) was initially effective in 83%;<sup>153</sup> half had osseous or articular infection. Seven of the 30 patients relapsed after treatment ranging in duration from 6 to 18 months. Thus, prolonged treatment for 12 or more months is needed.

Sulfonamides, terbinafine and potassium iodide are not recommended for this form of infection. In patients in whom osteoarticular infection is part of a more widespread disseminated infection which is potentially life-threatening, initial therapy with either a lipid formulation of amphotericin B, given at a dose of 3–5mg/kg daily, or amphotericin B deoxycholate, administered at a dose of 0.7–1.0 mg/kg daily, is warranted. Therapy may subsequently be changed to itraconazole, once the patient has favorably responded and is clinically stable.<sup>153a</sup> Surgical debridement or synovectomy may be indicated in some cases, particularly for those with tenosynovitis or carpal tunnel syndrome. A case of apparently successful total knee arthroplasty after medical treatment of sporotrichal arthritis has been reported.<sup>194</sup>

## ***Aspergillus* species**

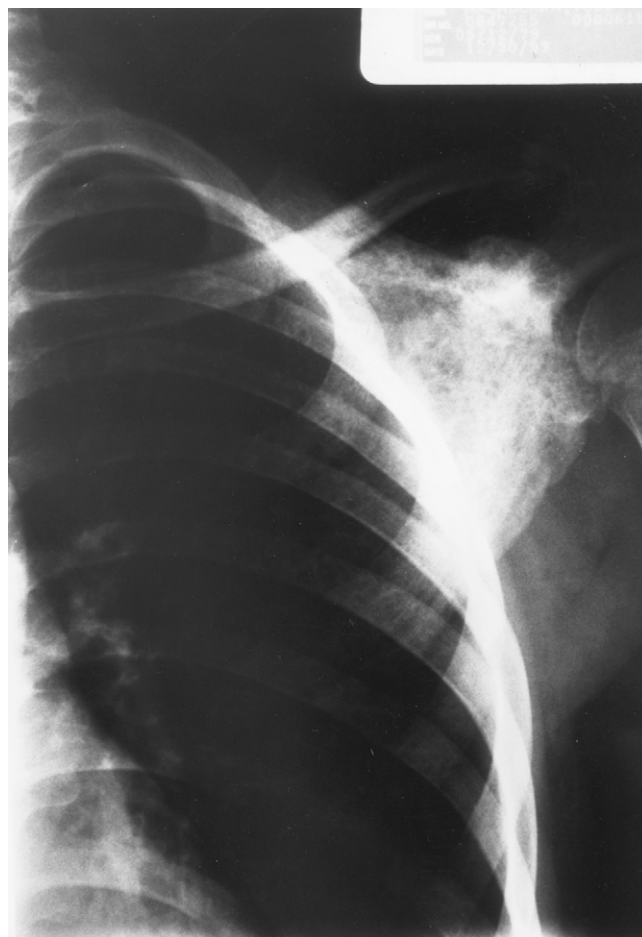
### **Bone and joint disease**

*Aspergillus* infection of the skeletal system usually results from the spread of infection from contiguous sites of thoracic or oronasopharyngeal infection or hematogenous dissemination of the organism to bone, vertebral disk space and, rarely, to joints.<sup>10,154</sup> The hyphal organisms colonize the oropharyngeal, nasal, and bronchial mucosa, where the spores germinate in the mucous layer. They then gain entry into tissue, invading vascular structures, causing thrombosis and extensive necrosis. Patients with this infection usually have reduced neutrophil function and numbers<sup>155</sup> or are otherwise profoundly immunocompromised, such as those who have transplanted organs,<sup>156</sup> AIDS,<sup>157</sup> hematogenous or lymphoproliferative malignancies, or those who have received immunosuppressive therapy.<sup>158</sup>

In a review of 27 patients with *Aspergillus* osteomyelitis, 11 (41%) had received antibiotics, 11 (41%) corticosteroids, and five (19%) both corticosteroids and immunosuppressive therapies.<sup>159</sup> In six of the patients, osteomyelitis developed at or near the site of a surgical procedure, including two prosthetic hip infections, a sternal wound infection after cardiac bypass grafting, and three vertebral infections after aortic aneurysm repair and laminectomy. Such iatrogenic infections often occur in otherwise immunologically healthy adults.<sup>160</sup> Traumatic introduction of the organism can occur, as in the case of a heart transplant patient in whom tibial osteomyelitis developed at the site of a pretibial wound resulting from a fall during cardiac arrest.<sup>161</sup> Isolated osteomyelitis has also been reported in parenteral drug users.<sup>162</sup>

Extension of infection to the maxillofacial structures, sphenoid bones, mastoids, and basilar skull can occur<sup>163</sup> but approximately half of reported cases of *Aspergillus* osteomyelitis involve the vertebrae.<sup>164–167</sup> Of 17 cases of vertebral osteomyelitis, paraspinal abscess was noted in six, disk space involvement in five, and involvement of posterior spinal structures in three. The ribs, clavicles, and scapula are also occasionally infected (Fig. 25-8).

*Aspergillus* is a common cause of disseminated fungal infection in children with chronic granulomatous disease (see Fig. 25-8). In one review of 42 such children, five had a history of skeletal fungal infection, all due to *Aspergillus* (eight other skeletal infections were due to mycobacteria, *Serratia*, *Nocardia* or *Staphylococcus*).<sup>8</sup> The vertebrae were involved in three, the ribs in three, and the sternum in one, all due to extension of infection from the thoracic cavity.



**Figure 25-8** *Aspergillus fumigatus* infection of the scapula in a 5-year-old boy with chronic granulomatous disease. A cutaneously draining sinus formed over the scapula. The scapula and adjacent tissue required extensive debridement and surgical resection of bone.

The clinical presentation is varied, but most patients complain of pain and tenderness at the site and fever. Both the clinical and radiographic appearance of vertebral aspergillosis resemble tuberculosis.<sup>165,166</sup> Common symptoms of head and neck infection include periorbital cellulitis, conjunctivitis, proptosis, nasal discharge, headache, and epistaxis. Leukocytosis is present in a minority of patients, and erythrocyte sedimentation rates range between 33 and 135 (mean 83) mm/h.<sup>159</sup> Cultures of biopsy or surgical specimens are generally positive, but the characteristic acutely branching hyphae seen in biopsy specimens could be diagnostic of either *Aspergillus* or *Fusarium* species.

Isolated cases of joint space infection, as a result of either hematogenous dissemination<sup>168</sup> or the introduction of the organism during trauma or a surgical or arthroscopic procedure,<sup>154,159,169</sup> have been reported. Joint infection often also involves contiguous bone.

### **Management**

Most successful published experience in the treatment of osteoarticular infections due to aspergillus species have utilized amphotericin B, often in combination with a second agent, and

surgical debridement.<sup>170</sup> In one report, therapy with amphotericin B alone failed in all three patients with vertebral osteomyelitis, but subsequently they responded to surgical debridement and spinal stabilization in combination with this polyene.<sup>167</sup> In a retrospective review of 32 cases of spinal osteomyelitis, 14/20 patients (70%) who were treated with both medical and surgical treatment survived compared with 7/12 patients (58%) who received medical treatment alone.<sup>164</sup> Neurologic recovery was, however, greater in the second group (13% vs 40%, respectively). Not all patients, however, require extensive debridement. Apparent cure was obtained in nine patients with vertebral disk space infection due to *Aspergillus* who received medical therapy alone, despite the fact that seven were severely immunocompromised.<sup>171</sup> All nine patients received itraconazole for a median of 5.5 months (in addition to amphotericin B in seven patients and 5FC in six patients).<sup>171</sup> A key to successful therapy in these patients was the rapid recognition of infection and initiation of therapy. In patients with articular infection, extensive synovectomy should be considered.

Voriconazole is the initial treatment of choice, but secondary choices include a lipid formulation of amphotericin B, an echinocandin, itraconazole, voriconazole, and posaconazole.<sup>172,173,173a</sup> Amphotericin B is ineffective in the treatment of infections due to *A. terreus* and, possibly, some other species. 5-fluorocytosine, and occasionally rifampin, have been added to amphotericin therapy<sup>170</sup> based on in vitro data suggesting synergy and animal model work<sup>174,175</sup> but the availability of newer agents has likely made this approach obsolete. The success of voriconazole in other forms of aspergillosis makes this drug the likely treatment of choice for osteoarticular infection, as well.<sup>176</sup> Similarly, the increasingly frequent combination therapy involving, usually, voriconazole or a lipid formulation of amphotericin B together with an echinocandin may be considered in patients with osteoarticular infection, but there are no clinical data on which to draw a conclusion. In addition to these antifungal agents, interferon- $\gamma$  has been used as adjunctive immunomodulatory therapy in children with chronic granulomatous disease.<sup>177</sup>

## Zygomycetes

### Bone disease

Rhino-orbital-cerebral zygomycosis is an uncommon but potentially life-threatening and disfiguring infection that occurs most often in patients with poorly controlled diabetes and diabetic ketoacidosis or those who are otherwise severely immunosuppressed.<sup>178,179</sup> Corticosteroids, uremia, and possibly pregnancy may be inciting factors. The three most commonly implicated pathogenic genera of the family Mucoraceae are *Rhizomucor*, *Rhizopus*, and *Absidia*. Members of the families Cunninghamellaceae and Saksenaceae rarely cause invasive disease.<sup>180,181</sup> A case of sternal osteomyelitis due to *Apophysomyces elegans*, a lesser known member of the family Mucoraceae, has been described. The infection arose following a minor penetrating wound, failed to respond to therapy with amphotericin B alone, and required extensive surgical debridement.<sup>182</sup>

Infection occurs as a result of direct extension of infection from oronasopharyngeal mucosa to deeper structures, such as bone. Focal cutaneous infection with extension to bone also occasionally occurs.<sup>182,183</sup> The infection rapidly results

in extensive vascular destruction, thrombosis, and necrosis. Although uncommon, patients with zygomycosis of other bones, such as the femur and tibia, resulting from fungemia have been reported.<sup>181,184,185</sup> Hematogenous dissemination to bone marrow, without evident osteomyelitis, usually in patients with severe pulmonary zygomycosis, may rarely occur.

Rapid diagnosis is critical to the survival of the patient. Presenting signs and symptoms of head and neck infection are similar to those described for aspergillosis, including pain and swelling over the involved area, nasal stuffiness and headache; epistaxis occurs in a minority of patients. A black necrotic eschar or ulcer may be directly observed on nasal or oropharyngeal mucosa. Involvement of the orbit leads to periorbital cellulitis, visual disturbances, proptosis, and headache. Unfortunately, by the time the diagnosis has been established, more than two-thirds of patients are obtunded or comatose. Presenting symptoms include lethargy and facial swelling, decreased vision, dehydration, acidosis, facial nerve palsies, external ophthalmoplegia, nasal discharge, and internal ophthalmoplegia. Cavernous sinus thrombosis and central nervous system involvement are the most dreaded complications of this disease. MRI provides comprehensive information on the extent of soft tissues and bone infection.<sup>184</sup>

Although cultures are often positive and suggest the presence of tissue infection, the diagnosis is made by visualization of the organism in biopsy specimens.

Amphotericin B is the treatment of choice, and combined medical and surgical therapy, with aggressive debridement of infected tissue and bone, is most effective.<sup>184,186</sup> Despite the prompt initiation of aggressive therapy, the infection may prove difficult to control, and patients may succumb to their disease.

In forms of infection other than that involving bone or joint, amphotericin B, usually a lipid formulation, is still considered the treatment of choice by many clinicians, although posaconazole appears to be similarly effective. A partial or complete response to posaconazole therapy was achieved in 60% of patients in a retrospective review of 91 cases of zygomycosis (20% of whom had been receiving voriconazole at the time they developed breakthrough zygomycosis) and 60% survived.<sup>187</sup> In comparison, survival rates of 61% and 69% have previously been reported in zygomycete-infected patients treated with amphotericin B deoxycholate and lipid-associated formulations of amphotericin B, respectively.<sup>188</sup> Combined medical and surgical therapy, with aggressive debridement of infected tissue and bone, is most effective.<sup>182,186</sup> Despite the prompt initiation of aggressive therapy, the infection may prove difficult to control, and patients may succumb to their disease.

## Eumycetoma agents

### Bone and joint disease

Mycetoma is caused by both fungi and anaerobic actinomycetes; only the former will be discussed here. The disease most often occurs in patients who live in developing countries in tropical and temperate zones, such as Mexico, Sudan, and Senegal. A variety of fungi have been implicated, but the particular pathogen depends on the geographic location in which the infection was acquired. In the United States, *Pseudallescheria boydii* is the most frequently reported fungal agent of



eumycetoma.<sup>189</sup> Other major fungi responsible for this clinical entity include *Madurella mycetomatis*, *Leptosphaeria senegalensis*, *Madurella grisea*, *Acremonium* species, and *Pyrenochaeta romeroi*.<sup>190,191</sup> Dematiaceous fungi, the brown pigmented moulds, found throughout the environment on decaying wood and plant debris, rarely result in osteomyelitis, typically as the result of traumatic implantation of the organism and progressive mycetoma.<sup>192</sup>

A true mycetoma involves cutaneous and subcutaneous tissues, as well as bone with fistular tracts. Approximately 70% of these infections involve the foot (Madura foot), but the hands (12%) are also commonly affected. The wrists, knees, legs, thighs, and head and neck are less common sites of mycetoma.<sup>189,191,193</sup> After traumatic implantation of the organism into the cutaneous or subcutaneous tissues, a painless subcutaneous nodule forms, which gradually increases in size. Sinus tracts frequently develop, which may persist or appear to heal superficially, usually temporarily, and the infection eventually spreads to deeper structures, including bones and joints. Chronic disabling pain may result. This process evolves over a minimum of 3 months and for as long as many years.

Early radiographic changes include osteoporosis and osteolysis with loss of the cortical margin; gross destruction of bone with small cavities and calcification are late findings. On probing, a granule or clusters of granules may be seen inside focal abscesses. A granule or clusters of granules may be found in drainage specimens and serve as a means of distinguishing causes. For example, because of its propensity to produce melanin, *Madurella mycetomatis* often forms black granules.<sup>191</sup> Attempts to identify the causative agent, with either needle or surgical biopsy, should be undertaken before the initiation of treatment.

In general, both surgical and medical intervention is required for effective management of the patient with mycetoma. While ketoconazole has some efficacy and may be used in circumstances in which limited resources demand its use, itraconazole may be a more effective oral alternative. Amphotericin B may be useful, depending upon the etiology, but the need for prolonged therapy makes its definitive use problematic. Information regarding the use of other agents is limited.

## Miscellaneous mycoses

Although this text discusses the fungi that more commonly cause bone and joint disease, most of the 200 or so fungi pathogenic in man have been reported to cause musculoskeletal infection. Some of these more unusual infections include, for example, those due to *Alternaria* species, *Acremonium* species, *Bipolaris hawaiiensis*, *Cunninghamella bertholletiae*, *Exophiala jeanselmei*, *Exophiala spinifera*, *Fusarium solani*, *Penicillium marneffeii*, *Phaeoacremonium parasiticum*, *Pseudallescheria boydii*, *Saccharomyces* species, *Scedosporium prolificans*, and *Trichosporon beigelii*.<sup>5</sup> As with many skeletal infections due to fungi, the etiology is often recognized belatedly.

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# Fungal infections of the genitourinary tract

Jack D. Sobel

## *Candida* vulvovaginitis

### Epidemiology

Statistical data from Great Britain reveal a sharp increase in the incidence in *Candida* vulvovaginitis (CVV).<sup>1</sup> In the United States, *Candida* is now the second most common cause of vaginal infections. Seventy-five percent of women experience at least one episode of CVV during their child-bearing years, and approximately 40–50% of them experience a second attack. A small subpopulation of women has repeated, recurrent episodes of *Candida* vaginitis.<sup>2,3</sup> Information on the incidence of CVV is incomplete, since CVV is not a reportable entity. Collecting data is hampered by inaccuracies of diagnosis, both practitioner based and self-diagnosis, and finally using non-representative study populations.

*Candida* spp. may be isolated from the genital tract of approximately 20% of asymptomatic, healthy women of child-bearing age.<sup>4</sup> The natural history of asymptomatic colonization is unknown. DNA typing techniques capable of “fingerprinting” *Candida* isolates reveal long-term vaginal colonization with the same strain of *Candida* over months and years.<sup>5</sup> Several genetic, biologic, and behavioral factors are associated with increased rates of asymptomatic vaginal colonization with *Candida* (Fig. 26-1), including recent antibiotic use, pregnancy (30–40%), use of high estrogen-content oral contraceptives, and uncontrolled diabetes mellitus. Other contraceptive measures, including the intrauterine device, diaphragm, vaginal sponge, and spermicidal nonoxynol-9, may also act as risk factors for *Candida* colonization. Some evidence exists that sexual intercourse frequency may influence the incidence of CVV.<sup>6</sup> The rarity of *Candida* isolation in premenarchal girls and the lower prevalence of *Candida* vaginitis after menopause emphasize the hormonal dependence of the infection. *Candida* vaginitis virtually only occurs in elderly women in the presence of uncontrolled diabetes mellitus or associated with the use of exogenous estrogen replacement therapy.

### Pathogenesis

#### The organism

Between 85% and 90% of yeasts isolated from the vagina are *Candida albicans* strains.<sup>2</sup> The remainder are due to other species, the most common of which are *C. glabrata* and

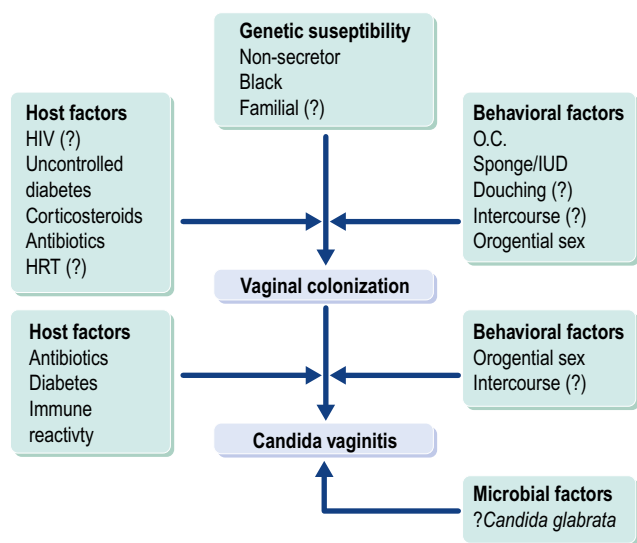
*C. tropicalis*. Non-*albicans* *Candida* species are capable of inducing vaginitis and are often more resistant to conventional therapy. There is some evidence of an increase in yeast vaginitis due to non-*albicans* *Candida* species, especially *C. glabrata*.<sup>7-9</sup> Risk factors for *C. glabrata* include diabetes, old age, and previous use of azole antimycotics.<sup>10</sup> In particular, the widespread use and abuse of over-the-counter antifungal agents and the now popular use of long-term maintenance fluconazole for recurrent CVV may be selective for relatively resistant *C. glabrata*.<sup>11,12</sup>

For *Candida* organisms to colonize the vaginal mucosa, they must first adhere to the vaginal epithelial cells. *C. albicans* adheres in significantly higher numbers to vaginal epithelial cells than do *C. tropicalis* and *C. krusei*. Germination of *Candida* enhances colonization and facilitates tissue invasion. Factors that enhance or facilitate germination (e.g., estrogen therapy and pregnancy) tend to precipitate symptomatic vaginitis, whereas measures that inhibit germination (e.g., bacterial flora) may prevent acute vaginitis in women who are asymptomatic carriers of yeast. Other virulence factors include proteolytic enzymes, secreted aspartyl proteinases mycotoxins, phospholipase elaboration, and iron use.<sup>13</sup> *Candida* organisms gain access to the vaginal lumen and secretions predominantly but not exclusively from the adjacent perianal area. This finding is borne out by several epidemiologic and typing studies. Orogenital sexual transmission may also facilitate genital colonization.<sup>6</sup> *Candida* vaginitis is seen predominantly in women of child-bearing age, and only in the minority of cases can a precipitating factor be identified to explain the transformation from asymptomatic carriage to symptomatic vaginitis.

#### Host factors

During pregnancy the clinical attack rate is maximally increased in the third trimester, but symptomatic recurrences are more common throughout pregnancy. It is generally thought that the high levels of reproductive hormones raise the glycogen content in the vaginal environment and provide an excellent carbon source for *Candida* growth and germination. The likely but more complex mechanism is that estrogens enhance vaginal epithelial cell avidity for *Candida* adherence, and a yeast cytosol receptor or binding system for female reproductive hormones has been documented. These hormones also enhance mycelium formation by the yeast cells. Low-estrogen oral contraceptives have not been found to cause an increase in





**Figure 26-1** Risk factors in the pathogenesis of *Candida* vaginitis. HIV, human immunodeficiency virus; HRT, hormone replacement therapy; O.C., oral contraceptive; IUD, intrauterine contraceptive device.

*Candida* vaginitis. Vaginal colonization with *Candida* is more frequent in diabetic women, and uncontrolled diabetes predisposes to symptomatic vaginitis. Glucose tolerance tests have been recommended for women with recurrent CVV; however, the yield is low, and testing is not justified in otherwise healthy premenopausal women.

Genetic predisposition to vaginal colonization with *Candida* has been suggested by Chaim et al,<sup>14</sup> who showed that women prone to CVV are significantly more likely to be genetic and phenotypic non-secretors of blood group antigens. The latter serve as buccal and vaginal epithelial cell membrane yeast receptors. In addition to blood group antigens anecdotally, strong family histories of susceptibility to CVV are often obtained. More scientific support is provided by recent mannose-binding lectin (MBL) studies. MBL, a component of the innate immune system, binds to mannose residues on *Candida* and promotes phagocytosis and complement activation.<sup>15</sup> Women with recurrent and chronic CVV (RCVV) have significantly reduced MBL levels.<sup>16</sup> Similarly, controlled cohort studies reveal that vaginal colonization with *Candida*, although high in human immunodeficiency virus (HIV)-negative high-risk behavior women, is significantly higher in HIV-positive women. Colonization does not appear to increase with progressive decline in CD4 cell count.<sup>17</sup>

Symptomatic CVV is frequently observed during or after use of systemic antibiotics.<sup>18,19</sup> Although no antimicrobial agent is free of this complication, the broad-spectrum antibiotics such as tetracycline, ampicillin, and cephalosporin are mainly responsible and act by eliminating the normal protective vaginal bacterial flora.<sup>20</sup> The natural flora provides a colonization resistance mechanism and prevents *Candida* germination. *Lactobacillus* species have been singled out as providing this protective function. Vaginal colonization rates increase from 10% to 30% following antibiotic administration and estimates of postantibiotic CVV average 25–33%.<sup>20</sup> Other unproved factors that anecdotally predispose to *Candida* vaginitis include the use of tight, poorly ventilated clothing

and nylon underclothing, which increases perineal moisture and temperature. Chemical contact, local allergy, and hypersensitivity reactions may also predispose to symptomatic vaginitis. No evidence confirms that iron deficiency predisposes to infection.

Oral and vaginal thrush correlate well with depressed cell-mediated immunity (CMI) in debilitated or immunosuppressed patients. This is evident in chronic mucocutaneous candidiasis and acquired immunodeficiency syndrome (AIDS). Accordingly, it might be anticipated that lymphocytes and CMI contribute to normal vaginal defense mechanisms, preventing mucosal invasion by *Candida*.<sup>21–24</sup> *Candida* antigen-stimulated peripheral blood mononuclear cells elaborate heat-stable peptides, possibly cytokines that inhibit yeast proliferation and germination. Candidates for this protective function include interferon- $\gamma$  and interleukin (IL)-2 as part of the Th1 cellular response.<sup>22</sup> Evidence exists of a compartmentalized vaginal anti-*Candida* T cell-protective immune response that functions independently of other mucosal sites, as well as systemic CMI.<sup>23–25</sup> Vaginal epithelial cells may provide yet another independent innate anti-*Candida* defense mechanism.<sup>26,27</sup>

## Pathogenesis of recurrent and chronic *candida* vaginitis

Careful evaluation of women with recurrent vaginitis usually fails to reveal any precipitating or causal mechanism (see Fig. 26-1). The intestinal reservoir theory is based on recovery of *Candida* on rectal culture in almost 100% of women with CVV and implies that repeated vaginal reintroduction from the perianal area occurs. Typing of simultaneously obtained vaginal and rectal cultures almost invariably reveals identical strains. This theory has been criticized because several authors demonstrated lower concordance between rectal and vaginal cultures in patients with recurrent and chronic *Candida* vaginitis.<sup>25</sup>

In a maintenance study of women with recurrent vaginitis receiving ketoconazole, recurrence of *Candida* vaginitis frequently occurred in the presence of negative rectal cultures for *Candida*.<sup>25</sup> Controlled studies using oral nystatin treatment, which reduces intestinal yeast carriage, failed to prevent recurrence of CVV. Penile colonization with *Candida* is present in approximately 20% of male partners of women with RCVV, and infected partners usually carry identical strains.<sup>28</sup> Sexual transmission of *Candida* is likely by intercourse and orogenital sex but is not thought to be the cause of recurrence in most cases. No single controlled study has shown that treatment of men with topical or systemic antimycotic agents prevents recurrence in women.

Vaginal relapse implies that incomplete eradication or clearance of *Candida* from the vagina occurs after fungistatic antimycotic therapy, although the latter may be sufficient to reduce the numbers of *Candida* in the lumen and alleviate signs and symptoms of inflammation. Organisms persist in small numbers in the vagina and result in continued carriage of the organism, and when host environmental conditions permit, the colonizing organisms increase in number and undergo mycelial transformation resulting in a new clinical episode.

RCVV, especially when caused by *C. albicans*, is rarely due to drug resistance; however, lack of susceptibility to azoles may be a factor in chronic *C. glabrata* infection.<sup>12</sup> Current theories

regarding pathogenesis of RCVV include qualitative and quantitative deficiency in the normal protective vaginal bacterial flora and, more importantly, an acquired potentially reversible antigen-specific deficiency in T lymphocyte functions that similarly permits unchecked yeast proliferation and germination. According to Witkin, reduced T lymphocyte reactivity to *Candida* antigen is the result of the elaboration by the patient's macrophages of prostaglandin E<sub>2</sub>, which blocks *Candida* antigen-induced lymphocyte proliferation, possibly by inhibiting IL-2 production.<sup>21,22</sup> Abnormal macrophage function could be the result of histamine produced as a consequence of local IgE *Candida* antibodies or a serum factor. Fidel et al failed to find corroborative evidence of protective T cell dysfunction in the vaginal mucosa and concluded that enhanced vaginal colonization is due to impaired vaginal epithelial cell function and that CVV supervened when yeast numbers exceed natural defenses, including polymorphonuclear leukocytes (PMNs).<sup>23,24</sup>

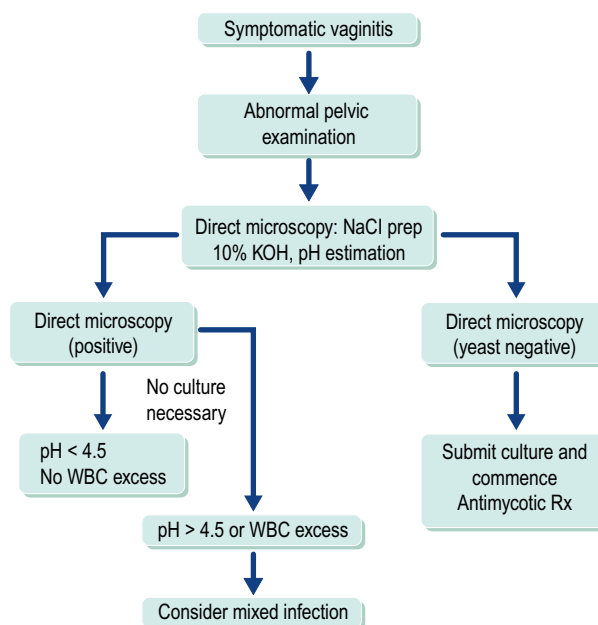
## Clinical manifestations

Symptoms and signs vary considerably in intensity from mild to severely incapacitating disease. The most frequent symptom is vulvar pruritus, which is present in virtually all symptomatic patients. Vaginal discharge is not invariably present and is frequently minimal. Although described as typically cottage cheese-like in character, the discharge may vary from watery to homogeneously thick. Vaginal soreness, irritation, vulvar burning, dyspareunia, and external dysuria are commonly present. Odor, if present, is minimal and non-offensive. Examination reveals erythema and swelling of the labia and vulva, often with discrete pustulopapular peripheral lesions. The cervix is normal, and vaginal mucosal erythema with adherent whitish discharge is present. Characteristically, symptoms are exacerbated in the week before the onset of menses, with some relief with the onset of menstrual flow. Clinical manifestations appear proportional to the microorganism load or population numbers with a less well-defined relationship between symptoms and morphotype of the infecting organism.<sup>29</sup>

## Diagnosis

None of the clinical manifestations is pathognomonic of CVV, hence clinical diagnosis must always be confirmed by laboratory methods. Most patients with symptomatic CVV may be readily diagnosed on the basis of vaginal pH estimation and microscopic examination of vaginal secretions. A wet mount or saline preparation has a sensitivity of 40–60%. The 10% KOH preparation is even more sensitive in diagnosing the presence of hyphal elements. A normal vaginal pH (4.0–4.5) is found in *Candida* vaginitis, and the finding of a pH in excess of 4.5 should strongly alert clinicians to the possibility of bacterial vaginosis, trichomoniasis or a mixed infection (Fig. 26-2).

Routine cultures in microscopy-positive patients are unnecessary; however, vaginal culture should be performed in a suspected patient with negative microscopy. Although vaginal culture is the most sensitive method available for detecting *Candida*, it should not be assumed when cultures are positive that *Candida* is invariably responsible for the vaginal symptoms. There is no reliable serologic technique for the diagnosis of symptomatic CVV. Newer diagnostic techniques



**Figure 26-2** Diagnostic investigation for suspected *Candida* vaginitis. WBC, white blood cell.

using DNA probes are now available and are of great value to practitioners who no longer avail themselves of microscopy. These new tests are expensive and offer minor advantage to physicians competent in the use of standard microscopy. PCR detection of *Candida* isolates is possible but is not available commercially.<sup>30</sup>

Differential diagnosis of CVV includes trichomoniasis and bacterial vaginosis from which CVV can be readily recognized using pH estimation and microscopy, although mixed infections occasionally occur. More difficult to separate, when patients with CVV have negative microscopy and normal vaginal pH, are chemical, irritant or allergic vulvovaginitis and vulvovestibulitis syndromes.

## Treatment

### Topical agents for acute *Candida* vaginitis

Antimycotics are available for local use as creams, lotions, aerosol sprays, vaginal tablets, suppositories, and coated tampons (Table 26-1). In the last decade, only one new commercial agent has been added to the armamentarium – reformulated butoconazole in a single-dose bioadhesive formulation. There is little to suggest that formulation of the topical antimycotic influences clinical efficacy.<sup>31</sup> Nystatin creams and vaginal suppositories achieve mycologic cure rates of approximately 75–80%.<sup>32</sup> Azole agents achieve slightly higher clinical and mycologic cure rates than do the polyenes (nystatin), at approximately 85–90%.<sup>31,32</sup> There is little evidence that any azole agent is superior to others.<sup>33</sup> Topical azoles are generally free of local and systemic side effects although the initial application is not infrequently accompanied by burning and discomfort. Moebius reported the occurrence of fever and influenza-like symptoms in patients using a high-dose terconazole regimen, which has been withdrawn.<sup>34</sup>

There has been a major trend toward shorter topical antifungal treatment courses with progressively higher antifungal drug doses, culminating in single-dose therapeutic regimens. Short courses and single-dose regimens have been shown to be effective for most of the azole and polyene antifungals in uncomplicated vaginitis.<sup>31,32,35,36</sup> In the United States, miconazole, clotrimazole, butoconazole, and tioconazole vaginal preparations are available without prescription as over-the-counter agents. Little is known as to whether their widespread consumption has been abused, associated with adverse effects, or contributed to antifungal drug resistance. A major concern, however, is the inappropriate use of antimycotics for self-diagnosed *Candida* vaginitis in which another cause or pathogen is responsible for symptoms.

#### Oral antimycotic agents

Ketoconazole (400 mg daily for 5 days) and itraconazole (200 mg daily for 3 days or 400 mg for 1 day) have largely been replaced by fluconazole (150 mg in a single daily dose). All have been shown to be highly effective in achieving clinical mycologic cure in acute *Candida* vaginitis.<sup>32,36-40</sup> Clinical results of oral therapy are at least as good as conventional topical antimycotic therapy. Several studies indicate that, given the choice, most women prefer oral therapy.<sup>41</sup> Women with more severe inflammation findings and symptoms should be given more than a single dose of fluconazole and a second or third dose is advised, given at 72-hour intervals.<sup>42</sup>

Any therapeutic advantage of oral therapy must be weighed against the potential for side effects and toxicity. Ketoconazole therapy is accompanied by gastrointestinal upset (10%) and rare anaphylaxis, but the major concern is the risk of hepatotoxicity, which occurs in approximately 1 in every 10,000–15,000 women treated.<sup>43</sup> Ketoconazole is now rarely used for CVV. Similar side effects appear to be much less frequent with the use of itraconazole and fluconazole. Drug interaction is not infrequent between azoles and a variety of commonly used agents. Ketoconazole and itraconazole should not be used together with the antihistamine agents terfenadine and astemizole.

Management of CVV during pregnancy is more difficult, because clinical response tends to be slower and recurrences are more frequent. Most topical antifungal agents are effective, especially when prescribed for longer periods of 1–2 weeks; however, single-dose therapy with clotrimazole has been shown to be effective during pregnancy.

Given the large armamentarium and different formulations of antifungal agents, it is now possible and desirable for clinicians to individualize therapy on the basis of objective clinical criteria such as severity of infection, history of frequent episodes in the past, and host characteristics, as well as taking into consideration the patient's preference for oral or intravaginal therapy. A useful classification of CVV now exists that should facilitate antifungal drug and regimen selection.<sup>44</sup> Uncomplicated CVV occurs in normal hosts, with mild or moderate severity infection, caused by *C. albicans* and in the absence of a history of recurrent disease. Uncomplicated infection that constitutes most symptomatic episodes responds well to oral vaginal therapy with all antimycotics, including short-course and single-dose regimens. Complicated CVV refers to severe infections, including those caused by relatively resistant non-*albicans* species of *Candida*, especially in women with a history

of physician-confirmed recurrent *Candida* vaginitis and those with underlying immunodeficiency. Complicated infections respond less well to short-course antimycotic regimens and required more prolonged antifungal therapy of at least 7-day regimens.<sup>44</sup> In addition, women with recurrent CVV will also require a long-term maintenance antifungal regimen.

#### Treatment of RCVV

The management of women with RCVV aims at control rather than cure. The diagnosis of RCVV must be confirmed, and reversible causes eliminated where possible. Unfortunately, in most women with RCVV, no underlying or predisposing factor is usually identified. RCVV requires long-term maintenance with a suppressive prophylactic regimen. Because of the chronicity of therapy, the convenience of oral treatment is apparent, and the best previous suppressive prophylaxis was achieved with daily low-dose ketoconazole, 100 mg daily for 6 months.<sup>45</sup> The benefit of successful suppressive therapy must be weighed against the potential toxicity of oral therapy. Low-dose ketoconazole is remarkably free of dose-dependent side effects but not from idiosyncratic toxic reactions such as hepatitis.<sup>43</sup> As an alternative to daily ketoconazole, weekly therapy with oral fluconazole 150 mg or topical clotrimazole (500 mg) is now recommended.<sup>12</sup> A multicenter prospective randomized control study confirmed the suppressive efficacy and safety of weekly fluconazole (success >90%).<sup>12</sup> In all reports of maintenance prophylaxis, cessation of antifungal prophylaxis is associated with resurgence of symptomatic infection in at least half of the women studied.<sup>12,45</sup> Dennerstein reported a reduced rate of recurrence in chronic CVV in 15 patients during a 3-month period of medroxyprogesterone acetate therapy.<sup>46</sup> Oral nystatin has little proven value in long-term prophylaxis.

RCVV is rarely the result of resistant vaginal yeast; however, in women who do not respond to conventional therapy, one may encounter unusual organisms (e.g., *Saccharomyces cerevisiae*, *C. glabrata* and *C. krusei*), which are known to have relatively higher minimum inhibitory concentrations to azoles. These patients respond to selected oral azoles, topical flucytosine, or topical boric acid.<sup>47,48</sup> Topical flucytosine use should be limited because of the tendency for the development of resistance.<sup>49</sup> The role of maintenance suppressive regimens for women with recurrent vaginitis due to *C. glabrata* is unknown. In patients with frequent recurrence of *C. glabrata* after an initial response to the aforementioned agents, a long-term regimen of topical nystatin in combination with ketoconazole or itraconazole can be prescribed after in vitro susceptibility tests indicate azole susceptibility.

#### *Candida* vaginitis and AIDS

Early reports indicated that CVV was more frequently encountered in women infected with HIV.<sup>50</sup> Moreover, it was frequently described as intransigent, chronic or recurrent.<sup>50,51</sup> CVV in at-risk women was considered to be an indicator of HIV infection, and women with recurrent episodes of CVV were encouraged to seek HIV testing. These reports were erroneous in that data from controlled studies were not available. All too often, diagnosis of RCVV was based on history alone without confirmation of diagnosis.<sup>50</sup> Most importantly,



RCVV is extremely common in healthy HIV-negative women. Accordingly, RCVV is not a useful predictive or discriminating diagnosis. CVV is common in women with early HIV infection and normal CD4 lymphocyte counts; however, this increase may be a reflection of the patient's lifestyle and sexual behavior rather than immunodeficiency. Although *Candida* colonization is more frequent in HIV-positive women, the modest increase in CVV in this population has little prognostic or diagnostic significance.<sup>52</sup> Moreover, clinical manifestations of CVV in HIV-positive women are not different or less responsive to therapy when compared to HIV-negative women.<sup>53,54</sup> Treatment of CVV in this population is identical to that advocated for HIV-negative women. There is some evidence that because of attendant inflammation, fissures, and microabrasions, CVV contributes to HIV transmission.<sup>55</sup>

### *Candida* balanoposthitis

Two forms of balanoposthitis (balanitis) are associated with *Candida* sp. Both types may be acquired sexually.

A true superficial but invasive infection occurs particularly in diabetic and uncircumcised men. It is characterized by intense pruritus, discomfort, erythema, and swelling, which is localized primarily to the glans but may extend to involve the penile shaft and scrotum. Cultures are invariably positive for *Candida* sp. Treatment consists of topical antimycotics or systemic azoles.

A milder but more common and particularly recurrent form of balanitis is also described in which penile cultures may be negative for *Candida*. Symptoms of local erythema or rash and pruritus typically appear soon after unprotected intercourse. Clinical manifestations are transient and often relieved by washing or topical steroids. They represent a proposed penile, cutaneous, immediate hypersensitivity reaction to the presence of *Candida* antigen in the vaginal secretions, often of symptomatic women. Cure requires eradication of *Candida* from the female source.

## Fungal infections of the urinary tract

Over the past 15 years there has been a marked increase in opportunistic fungal pathogens involving the urinary tract. *Candida* species are the most prevalent and pathogenic fungi in both the urinary and genital tracts of men and women.<sup>56-61</sup> The increased incidence of urinary tract fungal infections is primarily the result of expansion of the "at-risk" pool of patients together with the increased use of technologies that predispose to, or facilitate, fungal invasion of the urinary tract. The urinary tract becomes infected as a result of fungemia and hematogenous spread (i.e., funguria constitutes a manifestation of systemic fungal disease that may or may not be apparent at the time of detection of funguria) or ascending infection, usually in the presence of urinary obstruction (fungemia when it occurs is secondary to ascending pyelonephritis).

Epidemiologic clues are valuable in the diagnosis of opportunistic fungal infections of the urinary tract. *Candida* species are common causes of ascending infection in catheterized and obstructed urinary tracts, particularly in diabetic patients. Patients receiving immunosuppression therapy for renal transplantation are at risk for invasive fungal urinary tract

**Table 26-1** Therapy for vaginal candidiasis

Drug	Formulation	Dosage regimen
<b>Topical agents</b>		
Butoconazole*	2% cream	5 g × 3 d
Clotrimazole*	1 % cream	5 g × 7-14 d
	100 mg vag. tab.	1 tab. × 7 d
	100 mg vag. tab.	2 tab. × 3 d
	500 mg vag. tab.	1 tab. single dose
Miconazole*	2% cream	5 g × 7 d
	100 mg vag. supp.	1 supp. × 7 d
	200 mg vag. supp.	1 supp. × 3 d
	1200 mg vag. supp.	1 supp. single dose
Econazole	150 mg vag. tab	1 tab. × 3 d
Fenticonazole	2% cream	5 g × 7 d
Tioconazole*	2% cream	5 g × 3 d
	6.5% cream	5 g single dose
Terconazole	0.4% cream	5 g × 7 d
	0.8% cream	5 g × 7 d
	80 mg vag. supp.	80 mg × 3 d
Nystatin	100,000 U vag. tab.	1 tab. × 14 d
<b>Oral agents</b>		
Ketoconazole	400 mg bid	× 5 d
Itraconazole	200 mg bid	× 1 d
	200 mg	× 3 d
Fluconazole	150 mg	Single dose

\*Over the counter; vag., vaginal; tab., tablets; supp., suppository.

infection (UTI) caused by *Candida*, *Aspergillus*, and *Cryptococcus* species.<sup>62</sup> AIDS is associated with mucosal *Candida* infections but not candiduria; however, disseminated histoplasmosis and cryptococcosis, both common complications of AIDS, frequently involve the urinary tract.

With the exception of *Candida* species, none of the medically important fungi discussed in this chapter are common urinary pathogens and rarely are they responsible for the common clinical syndromes of urethritis, cystitis, and pyelonephritis. Nevertheless, all the aforementioned fungi can occasionally cause prostatitis, epididymitis, chronic bladder inflammation or ulceration, ureteric obstruction, and chronic renal disease (Table 26-2). In the absence of obstruction, fungal infections rarely cause renal insufficiency. Fungal infection should always be considered in the differential diagnosis of filling defects in the collecting system.

**Table 26-2** Urogenital tract involvement by invasive mycoses

	Epididymis	Testis	Prostate	Bladder	Kidney	Penis/ cutaneous
Blastomycosis	+	+	+++	+	+	+
Histoplasmosis	+	+	++	+	++	++
Coccidioidomycosis	+	+	+	+	++	+
Aspergillosis	+	+	+	+	+++	+
Cryptococcosis	+	+	+++	+	+++	+
Candidiasis	+	+	+++	++++	++++	++

## Urinary candidiasis

### Epidemiology

*Candida* microorganisms frequently exist as saprophytes on the external genitalia or urethra; however, yeasts in measurable quantities are found in <1% of clean voided urine specimens.<sup>63</sup> The overall frequency of *Candida* infections in hospitals has increased by 200% to 300% in the last decade, such that in a general hospital 5% of urine cultures may yield *Candida* species, and in tertiary care centers *Candida* species account for approximately 10% of all urinary isolates.<sup>58,64</sup> Platt et al, investigating nosocomial UTIs in patients with indwelling bladder catheters, concluded that 26.5% of infections were caused by fungi.<sup>57</sup> Most positive cultures are isolated or transient findings of little significance and represent colonization rather than true infection, and less than 10% of candidemias are the consequence of candiduria; nevertheless, *Candida* UTIs have emerged as important nosocomial infections.<sup>58,59,61</sup> Candiduria is especially common in the intensive care unit (ICU), and may represent the most common urinary infection in surgical and neonatal ICUs.<sup>58,59</sup>

### Microbiology

Although *Candida albicans* is the most common species isolated from the urine, in contrast to oral, esophageal, and vaginal candidiasis, non-*albicans* species of *Candida* account for almost half of the *Candida* urinary isolates.<sup>58,65,66</sup> *Candida glabrata* accounts for 25–35% of infections and other *Candida* species for 8–28%, including *C. tropicalis*, *C. krusei*, and *C. guilliermondii*. Unusual species are especially common in hospitalized patients, often diabetic patients, with long-term indwelling bladder catheters. Mixed infections due to more than one *Candida* species are not infrequent, as is concomitant bacteriuria.

### Pathogenesis

*Candida* infections of the urinary tract infrequently occur in the absence of predisposing factors or in normal hosts (Table 26-3). Most infections are associated with the use of indwelling urinary devices including Foley catheters, internal stents, and percutaneous nephrostomy tubes.<sup>67</sup> Diabetic patients have

**Table 26-3** Risk factors for *Candida* urinary tract infection (UTI)

	Route	Risk factors
Renal candidiasis	Hematogenous (anterograde)	Neutropenia (prolonged), intravascular drug use, burns, recent surgery (abdominal, thoracic), systemic infection
<i>Candida</i> lower UTI	Ascending (retrograde)	Foley catheter, female gender, extremes of age, instrumentation, diabetes mellitus, obstruction/stasis, recent antibacterial therapy, recent bacterial UTI, urinary stent, nephrostomy tube, renal transplantation
<i>Candida</i> pyelonephritis	Ascending	Diabetes, obstruction/stasis, instrumentation, postoperative, nephrostomy tube, ureteral stent, nephrolithiasis

an increased overall risk of UTIs, including bacterial infections, but especially those caused by fungi.<sup>57</sup> *Candida* growth in urine is enhanced when urinary levels of glucose exceed 150 mg/dl. Diabetic women have higher vaginal perineal and periurethral *Candida* colonization rates. Diabetic patients are also at risk because of impaired phagocytic and fungicidal activity of neutrophils associated with insulin deficiency; however, the dominant predisposing factor to candiduria is increased instrumentation, urinary stasis, and obstruction secondary to autonomic neuropathy.<sup>68</sup>

Antibiotic therapy plays a critical role in the pathogenesis of candiduria, the latter almost always emerging during or immediately after antibiotic therapy. No antibiotic appears

exempt from this complication, although broad-spectrum agents provide higher risk, as does the prolonged use of antibiotics. By suppressing susceptible autochthonous bacterial flora in the gastrointestinal and lower genital tract, antibiotic use results in the emergence of fungi colonizing these epithelial surfaces with ready access to the urinary tract, especially in the presence of indwelling bladder catheters.

Most lower UTIs are caused by retrograde infection from an indwelling catheter and genital or perineal colonization. The upper urinary tract may rarely become involved by means of ascending infection and then usually only in the presence of urinary obstruction, reflux, or diabetes.

Most cases of renal candidiasis occur not as a result of ascending spread from the lower urinary tract but as a consequence of hematogenous seeding of the renal parenchyma.<sup>69,70</sup> *Candida* species have a special tropism for the kidney. An autopsy study performed by Lehner documented that 90% of the patients dying with disseminated candidiasis had renal involvement, although renal infection (candidiasis) may occur as an isolated site of metastatic spread, especially after transient candidemia.<sup>70</sup> Autopsy studies demonstrate multiple abscesses in the renal interstitium, glomeruli and peritubular vessels, with not infrequent papillary necrosis and rarely complicated by emphysematous pyelonephritis.

## Clinical features

Most patients with candiduria are asymptomatic. Patients who have an indwelling bladder catheter most often are colonized rather than infected with a *Candida* species. Hospitalized candiduric patients with constitutional or systemic symptoms usually have a co-existent alternative cause of symptoms. Clinical manifestations caused by *Candida* infection depend on the site of infection. Patients with *Candida* cystitis have signs and symptoms of bladder irritation, including frequency, dysuria, urgency, hematuria, and pyuria. Cystoscopy reveals soft, pearly white, slightly elevated patches that resemble deposits of coagulated milk, as well as hyperemia and inflammation of the bladder mucosa.<sup>71</sup> Most symptomatic patients with *Candida* cystitis are not catheterized, and the converse also applies.

Ascending infection, although rare, may result in *Candida* pyelonephritis characterized by fever, leukocytosis, rigors, and costovertebral angle tenderness.<sup>72</sup> Ultrasonography and computed tomography scanning are useful in diagnosing an intrarenal and perinephric abscess. Excretory urography may reveal ureteropelvic fungus balls with or without accompanying papillary necrosis.<sup>73</sup> Ascending infection with *Candida* species uncommonly causes candidemia, with 3–10% of episodes of candidemia being secondary to candiduria.<sup>74</sup> When candidemia occurs, it invariably complicates anatomic obstruction, manipulation, or a urologic procedure.

Fungal bezoars may develop anywhere in the urinary drainage system but most commonly are found in the pelvis or upper ureters.<sup>73</sup> These fungal balls fortunately are rare, and their presence is suggested by signs of ureteral obstruction associated with candiduria. When bilateral, fungal bezoars may induce obstruction sufficient to cause azotemia. Obstruction may be intermittent or passage of the bezoars may result in renal colic or the passage of “soft” stones. Excretory urography or retrograde pyelography reveals filling defects in the

collecting system. Fungus balls in the urinary tract have also been described with aspergillosis and Zygomycetes.

Renal candidiasis secondary to hematogenous spread represents a systemic infection usually accompanied by fever and other constitutional manifestations of sepsis. Concomitant positive blood cultures may be obtained; however, often when the diagnosis of renal candidiasis is considered, blood cultures are no longer positive, causing considerable difficulty in establishing a diagnosis. Manifestations of disseminated candidiasis, including skin rash and endophthalmitis, may be present. Most patients with candiduria secondary to renal candidiasis are febrile but lack other clinical manifestations that indicate renal involvement other than variable reduction in renal function. Accordingly, finding candiduria may be the only clue to the diagnosis of invasive and disseminated candidiasis.<sup>75</sup>

## Diagnosis

Isolation of *Candida* sp. from a urine sample may represent contamination, colonization, or superficial or deep infection of the lower or upper urinary tract.<sup>58,76</sup> Contamination of the sample is particularly common in women with vulvovaginal colonization. Contamination can usually be excluded by repeating the urine culture with special attention to proper collection techniques. In fact, two consecutive positive isolates of *Candida* are essential before initiating antifungal therapy.

Differentiating infection from colonization of the urinary tract may be extremely difficult, if not impossible, in some patients. This is particularly so in catheterized subjects, and one often relies on accompanying clinical manifestations. Unfortunately, clinical features are not specific, and in critically ill patients in intensive care units, fever and leukocytosis may have several other sources. The presence of pyuria has not been shown to be helpful in differentiating infection from colonization. Most patients with significant candiduria have pyuria; however, the latter is difficult to interpret in the presence of an indwelling catheter, which may itself lead to pyuria from mechanical irritation of bladder mucosa and because of concomitant bacteriuria.

Quantitative urine colony counts have limited value in separating infection from colonization but only in the absence of a Foley catheter. The presence of the latter negates the value of quantitative cultures. In non-catheterized patients, some consider the mere presence of a *Candida* sp. in the urine, irrespective of count, to represent true infection. In contrast, Kozinn et al showed that counts of greater than 10,000–15,000/ml of urine were associated with infection.<sup>65</sup> Although only a minority of patients with high colony counts have true infection, it is rare for a patient with invasive disease of the kidney, renal pelvis, or bladder to have low colony counts. Renal candidiasis is rarely reported with a colony count of  $<10^3$ /ml. Thus considerable overlap occurs, and quantitative cultures are not the final determinant in therapeutic decision making, and similarly negative urine cultures cannot be used to exclude renal candidiasis.

After candiduria is deemed to represent infection, the challenge to the clinician is to localize the source or anatomic level of infection. Localization is critical in the management of candiduria. No useful test exists to differentiate *Candida* invasion of kidneys from the more frequent lower tract infection. The only specific or pathognomonic finding in renal candidiasis is



the detection of *Candida* hyphae or pseudohyphae enmeshed in a hyaline or granular tubular cast or urine microscopy. Unfortunately, this is a rare finding and, as is the case with quantitative cultures, fungal morphology on microscopy and pyuria have little value in localizing infection. Indirect non-specific evidence of upper tract infection is suggested by declining renal function, constitutional features, and radiographic findings on computed tomography scans and ultrasonography. Serologic tests for *Candida* antigens in parenchymal invasion remain insensitive and elusive. Amphotericin B irrigation initially used therapeutically has also been advocated as a diagnostic test; however, it has not been validated and is rarely used.<sup>77</sup>

## Management of candiduria (Table 26-4)

### Asymptomatic candiduria

Asymptomatic colonization with *Candida* is by far the most common syndrome associated with isolation of *Candida* species from the urine. No specific antifungal therapy is required for this condition. The natural history of asymptomatic candiduria is such that candiduria may be transient only and, even if persistent, uncommonly results in serious morbidity. The issue is not whether antifungal therapy, either systemic with amphotericin B or fluconazole, as well as local amphotericin B irrigation, can actually eliminate candiduria. Many studies have shown that local or systemic therapy can achieve this end result.<sup>78-81</sup> However, there is no evidence that patients benefit from therapy, and relapse is frequent. The risk of invasive complications is small.<sup>74</sup> A multicenter study conducted by the Mycoses Study Group found that in catheterized subjects, removal of the catheter and discontinuation of antibiotics eliminated the candiduria in approximately 40% of patients.<sup>82</sup> Not all experts agree that persistent asymptomatic candiduria in catheterized patients can be ignored.<sup>72</sup> In contrast, persistent candiduria in non-catheterized subjects should be investigated, because the likelihood of obstruction and stasis is relatively high. Persistent asymptomatic candiduria in an afebrile neutropenic patient merits both investigation to exclude the possibility of hematogenous renal candidiasis and empiric antifungal therapy.

Patients with known asymptomatic candiduria in whom urologic instrumentation or surgery is planned should have the candiduria eliminated or suppressed before and during the procedure to avoid the risk of invasive candidiasis and candidemia. Successful elimination can be achieved through amphotericin B or miconazole bladder irrigation, or with systemic therapy with amphotericin B, flucytosine or fluconazole. In the past, asymptomatic candiduria in renal transplant recipients was considered justification for antifungal therapy based upon the perceived risk of a destructive process in the kidney graft. However, a recent large study of posttransplant patients failed to show excessive morbidity associated with candiduria or any benefit from eradication of candiduria. Accordingly, most experts advise against therapy of asymptomatic candiduria in renal transplant recipients.<sup>83</sup>

### *Candida* cystitis

Symptomatic cystitis requires treatment with either amphotericin B bladder instillation (50 µg/dl) or systemic therapy, once more using intravenous amphotericin B, flucytosine or azole agents.<sup>73-82,84,85</sup> In general, amphotericin B bladder

**Table 26-4** Treatment of candiduria

Indications	Method
Symptomatic patients with definite or probable (? possible) systemic candidiasis (hence renal candidiasis)	Systemic antifungals IV AMB IV fluconazole
Symptomatic urinary tract infection	
Pyelonephritis	Systemic antifungal IV/PO fluconazole IV AMB
Lower tract	Systemic antifungal Oral fluconazole IV single dose AMB
Nonlocalized	Local AMB irrigation Systemic antifungal
Complicated	Systemic antifungal and drainage/ change or irrigation of nephrostomy tubes
Asymptomatic candiduria	
Rarely indicated except After renal transplant Preoperative urology Neutropenia	Systemic antifungal
Consider in presence of upper tract obstruction	
AMB, amphotericin B.	

irrigation is used less frequently as it is labor intensive and because of the availability of fluconazole.<sup>86</sup> Of the azole class, ketoconazole, itraconazole and voriconazole are poorly excreted in the urine, and there is limited, suboptimal clinical experience.<sup>87</sup> In contrast, fluconazole is water soluble, well absorbed orally, and >80% is excreted unchanged in the urine, achieving high urinary concentrations with documented clinical efficacy.<sup>88, 89</sup> Single-dose IV amphotericin B, 0.3 mg/kg, has also been shown to be highly efficacious in the treatment of lower UT candidiasis with therapeutic urine concentrations for a considerable time after the single dose of amphotericin B administration.<sup>90</sup> This regimen may be preferable for resistant fungal species. Most non-catheterized patients are conveniently managed with oral fluconazole but as this is a complicated infection, therapy should be continued for at least 7 days, dose 200–400 mg/day.

### Ascending pyelonephritis and *candida* urosepsis

Invasive upper tract infection requires systemic antifungal therapy and immediate investigation and visualization of the urinary drainage system to exclude urinary obstruction, papillary necrosis, and fungus ball formation. The most widely

accepted therapy is intravenous amphotericin B, 0.6 mg/kg/day, providing broad spectrum and urinary excretion.<sup>91</sup> Duration of therapy depends on the severity of infection, presence of candidemia, and response to therapy, in general 1–2 g total dose. As an alternative to amphotericin B, systemic therapy with fluconazole, 5–10 mg/kg/day (IV or oral), offers an effective and less toxic therapy.<sup>92</sup> Because fluconazole is excreted unchanged into the urine, frequently co-existent severe renal failure may result in subtherapeutic concentrations of fluconazole in tubular urine and at more distal sites. Accordingly, systemic doses of fluconazole should not be reduced in renal failure and postrenal candiduria. The echinocandin class of drugs (caspofungin, micafungin and anidulafungin) achieve low urinary concentrations only and accordingly are of little value for superficial urinary infections. However, when infection is invasive and involves the renal parenchyma, these candidacidal agents are useful, especially when dealing with non-albicans *Candida* species. Infection refractory to medical management should be treated surgically with drainage or, in cases of a non-viable kidney, nephrectomy. An obstructed kidney with hydronephrosis requires a percutaneous nephrostomy.

The management of ureteral fungus balls depends on the extent, site, and severity of infection. In some cases, bezoars spontaneously lyse or become dislodged during placement of ureteral stents.<sup>93</sup> In many cases, upper tract external drainage by means of a nephrostomy tube must be combined with local amphotericin B or fluconazole irrigation. Occasionally the fungal bezoars must be removed surgically.

#### Renal and disseminated candidiasis

Management of renal candidiasis secondary to hematogenous spread is essentially that of systemic candidiasis, including IV amphotericin B, 0.6 mg/kg/day, IV voriconazole 4 mg/kg bid, IV fluconazole, 400 mg/day, or any of the echinocandins. Dosage modification for azoles is necessary in the presence of severe azotemia. Prognosis depends on correction of underlying factors (i.e., resolution of neutropenia or removal of responsible intravascular catheters). Systemic candidiasis requires prolonged therapy of approximately 4–6 weeks' duration.<sup>91,92</sup>

## Rare fungal infections of the genitourinary tract

Accompanying the reports of increased superficial, deep, and systemic fungal infections, it is apparent that in the severely immunocompromised host, any and every fungal species can cause serious, if not life-threatening, disease.<sup>56,84,94,95</sup> This applies to virtually all fungal species, regardless of lack of demonstrable pathogenicity in the competent host (Table 26-5). Accordingly, the genitourinary tract may become involved at any anatomic level as a result of hematogenous spread. Fungal organisms that infrequently cause genitourinary tract infection include *Trichosporon* spp. (*asahi*) and *Blastoschizomyces capitatus*. They are best described as causing both systemic infection and localized infection in the urinary tract.<sup>96</sup> Resistant symptomatic cystitis due to *T. beigelii* after bladder instrumentation was reported in an elderly man. Symptoms responded rapidly to oral fluconazole.<sup>96</sup> *T. beigelii* has also been found as a contaminant of urinary drainage systems in a group of patients in an intensive care unit.<sup>56</sup>

**Table 26-5** Rare fungal infections of the genitourinary tract

Zygomycosis
Paracoccidioidomycosis
Geotrichosis
Sporotrichosis
Trichosporonosis
<i>Paecilomyces</i> infection
<i>Hansenula fabionii</i> infection
<i>Penicillium</i> species infection

## Cryptococcuria

Both symptomatic and asymptomatic cryptococcal UTIs can occur not only in AIDS patients but in patients with other immunocompromising conditions. In systemic cryptococcosis, cryptococcuria may occur as an early event preceding clinically evident meningitis.<sup>97</sup> It may co-exist with meningitis (30–40%) and, in this case, is a poor prognostic factor indicative of widely disseminated disease.<sup>98</sup> It may occur after apparently successful antifungal therapy and may be a source from which systemic infection can relapse.<sup>99</sup> Finally, isolated cryptococcal urinary tract infection can exist in the absence of systemic infection. However, the fact that systemic recurrence, meningitis, and death have occurred after relapse from a urinary source indicates that even in the absence of pulmonary or meningeal cryptococcosis, cryptococcuria is not benign. Hence, patients seen with cryptococcuria should be evaluated for systemic and meningeal infection, and their genitourinary tracts should also be investigated.

Clinical infection of the genitourinary tract may take three forms.<sup>100</sup>

- Pyelonephritis is a rare syndrome which is clinically indistinguishable from bacterial pyelonephritis. Most cases of kidney infection by cryptococcus are, however, silent or asymptomatic and discovered at autopsy or after the discovery of asymptomatic cryptococcuria. Only 30 cases of renal cryptococcal disease have been reported.<sup>101</sup> In these autopsy studies, occult renal involvement was found together with other manifestations of disseminated cryptococcal disease. Virtually all patients with symptomatic clinical pyelonephritis had some degree of immunosuppression at the time of diagnosis (e.g., steroids, diabetes, lymphoma).
- The most common clinical syndrome is cryptococcal prostatitis (see prostatitis section).
- Some patients with cryptococcuria will have no focal infection, identified by clinical signs, cultures, radiographs, or biopsy (i.e., occult cryptococcal UTI).

Most focal and occult involvement occurs without an increase in serum cryptococcal antigen titer. Nevertheless, occult infection with isolated cryptococcuria and no localizing signs must be seen as a marker of disseminated disease. This conclusion is supported by autopsy studies in patients with disseminated

cryptococcal infection, in which 26–51% of patients have renal foci of infection.<sup>101</sup> Treatment of symptomatic or asymptomatic cryptococcuria requires systemic antifungal agents, including IV amphotericin B, 0.7 mg/kg/day, or fluconazole, 5–10 mg/kg/day.

Cryptococcuria has increased in frequency since the onset of the AIDS epidemic and is less likely to be occult or asymptomatic.<sup>102</sup>

## Fungal prostatitis

Fungal infections of the prostate are by no means rare.<sup>56,62,103–113</sup> Fungal prostatitis may result from local inoculation (*Candida* and *Trichosporon* species) by contaminated or infected urine or from hematogenous spread (blastomycosis, histoplasmosis, coccidioidomycosis, aspergillosis, cryptococcosis, candidiasis, and zygomycosis). Frequently prostatic involvement by fungi is chronic, asymptomatic, and discovered at autopsy.

*Candida* species are the fungi that most commonly infect the urinary tract and hence the most common cause of prostatitis, followed by blastomycosis and cryptococcosis. Risk factors for *Candida* prostatitis are similar to those of UTI, especially diabetes mellitus, antibiotic administration, indwelling catheters, and anatomic abnormalities. In reality, given the high prevalence of candiduria, especially in catheterized patients, *Candida* abscesses of the prostate gland are extremely rare.

Acute prostatitis due to *Candida* species is rare, presenting with fever, constitutional findings, perineal pain, discomfort, urinary bladder irritative symptoms, and possibly urinary obstruction. The latter is more likely in the presence of a *Candida* prostatic abscess.<sup>113</sup> In most cases, urine cultures for *Candida* are positive, although rare instances of sterile urine have been reported. The presence of an abscess is confirmed by transrectal ultrasonography or computed tomography scan. In addition to systemic antifungal therapy (see urinary candidiasis), focal supuration requires drainage, either by the percutaneous route or occasionally by performing a transurethral prostatectomy.

Most of the non-*Candida* prostatic infections occur as a result of hematogenous dissemination, especially *Blastomyces dermatitidis*, which has a predilection for the prostate gland. Clinical features are identical for all the invasive mycoses. The diagnosis of chronic fungal prostatitis is usually entertained when symptomatic patients have laboratory signs of urinary inflammation but negative bacterial cultures (i.e., pyuria or increased leukocytes in expressed prostatic secretions or ejaculate). A negative fungal culture of urine or secretions should, however, not exclude the diagnosis of chronic fungal prostatitis given the pathology of granulomatous fungal prostatitis.

Cryptococcal infection of the prostate most commonly occurs as part of a disseminated process and may accompany pulmonary disease or meningitis. Most cases of cryptococcal involvement of the prostate are only diagnosed at autopsy. Although usually asymptomatic, the prostate gland has emerged as a potential site of relapse of cryptococcosis after seemingly successful treatment of AIDS.<sup>99</sup> In one series of AIDS-related cryptococcosis, *C. neoformans* was grown from urine in 9/41 patients after completion of a full course of amphotericin B, with fungi still evident on microscopic examination of expressed prostatic secretions.<sup>99</sup> Similar findings have been observed by Bailly et al<sup>105</sup> and by Staib et al<sup>114</sup> with positive urine and semen cultures. Hence the prostate serves as a reservoir

(i.e., focus from which infection is not eradicated and from which dissemination can occur).

Prostatic abscesses due to *C. neoformans* most commonly occur in immunocompromised hosts presenting with acute onset of dysuria, urinary frequency and hesitancy, accompanying fatigue, nausea and fever.<sup>115</sup> Physical examination may be surprisingly normal, usually revealing variable prostatic enlargement only. Clinically apparent prostatitis or abscess is more likely to be diagnosed in patients with AIDS.

Although the mainstay of treatment remains IV amphotericin B, often in combination with flucytosine, by virtue of its oral convenience, relative lack of toxicity, penetration, and efficacy in UTIs fluconazole has become the long-term treatment of choice.<sup>116,117</sup> Nevertheless treatment failures with fluconazole have been reported.<sup>105</sup>

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# Fungal infections of the respiratory tract

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## Introduction

Fungal infections of the respiratory tract are important causes of morbidity and mortality in immunocompromised patients. Among such patients are those receiving cytotoxic chemotherapy for neoplastic diseases, those undergoing hematopoietic stem cell transplantation (HSCT) or organ transplantation, and those afflicted with the acquired immune deficiency syndrome. As the armamentarium of immunosuppressive medications available to treat a variety of illnesses has evolved, the number of patients vulnerable to fungal infections has greatly expanded. For example, patients with autoimmune diseases such as refractory immune-mediated thrombocytopenia purpura and systemic lupus erythematosus are often treated with immunosuppressive agents and are thus at risk of developing fungal infections. In addition, patients receiving corticosteroid therapy are susceptible hosts. Finally, patients not typically classified as immunocompromised, including critically ill patients in an intensive care unit setting and premature infants, are also prone to fungal infections of the respiratory tract.

Invasive aspergillosis has been reported with increased frequency in parallel with this expanding population of immunocompromised patients. Other filamentous fungi, such as *Fusarium* spp., zygomycetes, and *Pseudallescheria boydii*, are reported with increasing frequency, particularly in patients with quantitative or qualitative defects in neutrophils. Endemic mycoses, including those due to *Histoplasma capsulatum* var. *capsulatum*, *Coccidioides immitis*, and *Penicillium marneffei*, have also increased in frequency in immunocompromised hosts in respective geographic regions (Table 27-1).

Patients who develop fungal infections of the respiratory tract often present with an array of non-specific symptoms that may mimic viral or bacterial infections. These symptoms can be mild or severe, ranging from low-grade fever, rhinorrhea, cough, and rash to dyspnea, hemoptysis, and shock. While these symptoms can be chronic and indolent, they can also progress rapidly in an immunocompromised host. Thus, early intervention is often crucial in effectively treating fungal infections.

Although dramatic strides in the diagnosis and treatment of respiratory fungal diseases have been made in recent years, it remains imperative for the clinician to maintain a high index of suspicion for the possibility of a pulmonary fungal infection when evaluating an ill, immunocompromised patient. This chapter will review the current approaches to the diagnosis, treatment, and prevention of fungal infections of the respiratory tract.

## Aspergillosis

### Classification of aspergillosis of the respiratory tract

Pulmonary aspergillosis may be classified as allergic, saprophytic or invasive<sup>1</sup> (Table 27-2). The allergic conditions induced by *Aspergillus* are further classified as involving the alveoli (extrinsic allergic alveolitis), the airways (extrinsic asthma and allergic bronchopulmonary aspergillosis (ABPA)) or the paranasal sinuses (allergic *Aspergillus* sinusitis).<sup>2-6</sup> Aspergilloma best typifies saprophytic processes of the lung, such as those involving cavities due to pulmonary tuberculosis, sarcoidosis, bronchiectasis, pneumocystosis, and cystic fibrosis.<sup>7</sup> Invasive aspergillosis, often presenting as a nosocomial infection of the respiratory tract in immunocompromised patients, develops as a bronchopneumonia or as invasive sinusitis.<sup>8-12</sup> Invasive pulmonary aspergillosis may be complicated by pulmonary hemorrhage, hemoptysis, invasion of contiguous structures or dissemination to extrathoracic organs. These disease states of pulmonary aspergillosis are not always clearly delineated entities; for example, a saprophytic pulmonary aspergilloma of a sarcoid cavity may become invasive when the patient is treated with corticosteroids for control of sarcoidosis.

### Clinical manifestations and diagnosis of aspergillosis of the respiratory tract

Recognition of aspergillosis of the respiratory tract requires skillful integration of the data derived from bedside evaluation, radiographic findings, and clinical microbiology. This

**Table 27-1** Common and emerging fungal pathogens causing respiratory mycoses

<b>Opportunistic fungi</b>
Hyaline Moulds <i>Aspergillus</i> spp. <i>Fusarium</i> spp. <i>Zygomycetes</i>
Dematiaceous Moulds <i>Pseudallescheria boydii</i> ( <i>Scedosporium apiospermum</i> ) <i>Scedosporium inflatum</i> <i>Bipolaris spicifera</i>
Yeasts <i>Cryptococcus neoformans</i> <i>Candida</i> spp. <i>Trichosporon asahii</i>
<b>Pathogenic dimorphic fungi</b>
<i>Histoplasma capsulatum</i> <i>Coccidioides immitis</i> <i>Blastomyces dermatitidis</i> <i>Penicillium marneffeii</i> <i>Sporothrix schenckii</i>

integrated approach toward the assessment of the clinical manifestations of the invasive, allergic, and saprophytic forms of aspergillosis facilitates an early diagnosis and initiation of therapy.

#### Allergic aspergillosis

Extrinsic allergic alveolitis due to *Aspergillus* occurs after repeated exposure in non-atopic workers to *Aspergillus* antigen in mouldy hay or grain, hence the terms “farmer’s lung” or “malt-workers lung.” Symptoms include cough, dyspnea, fever, chills, and myalgias within 8 hours of exposure. Patients report relief of symptoms after a weekend away from work only to be followed by recrudescence of symptoms upon returning to work on Monday. A chest radiograph may reveal interstitial infiltrates. Repeated exposure may lead to intractable pulmonary fibrosis.

The process of ABPA involves an allergic response to *Aspergillus* hyphae without direct tissue invasion by the organism. Bronchospasm in this process is thought to be mediated by an IgE (Type I reaction) immediate hypersensitivity, whereas the bronchial and peribronchial inflammation in ABPA appears to be induced by immune complex formation (Type III reaction). ABPA most often presents in children, adolescents, and young adults with asthma and evanescent, unexplained pulmonary infiltrates. Patients with ABPA also may describe expectoration of brown mucus plugs. These expectorated secretions consist of inflammatory cells, including eosinophils, as well as branching septate hyphae of *Aspergillus*. Patients with ABPA have elevated total IgE levels and often have proximal or central bronchiectasis.<sup>13</sup>

The link between sensitization and infection with *Aspergillus* and asthma is a subject of intensive study. In a recent

**Table 27-2** Classification of aspergillosis of the respiratory tract.

<b>Allergic</b>
Extrinsic allergic alveolitis Extrinsic asthma Allergic bronchopulmonary aspergillosis Allergic <i>Aspergillus</i> sinusitis
<b>Chronic or Saprophytic</b>
Pulmonary aspergilloma Sinus aspergilloma Chronic cavitary aspergillosis
<b>Invasive</b>
Acute invasive aspergillosis Necrotizing tracheobronchitis Invasive sinusitis Local extension to intrathoracic structures Disseminated aspergillosis Chronic necrotizing aspergillosis

prospective study of patients attending a chest clinic in northern India, 27% of asthmatics met criteria for ABPA.<sup>13</sup> There is also evidence demonstrating an association between fungal sensitization and asthma severity.<sup>14,15</sup> Whether the relationship is causative remains unknown, but the role of antifungal agents in the treatment of asthma patients who demonstrate fungal sensitization is currently being explored. One potential mechanism for asthma potentiation by *Aspergillus* is via activation of TLR-2, which upregulates the Th2 immune response and triggers pulmonary inflammation in vivo.<sup>16,17</sup>

Allergic *Aspergillus* sinusitis is found in immunocompetent, atopic patients with a history of repeated bouts of sinus congestion that have become more severe and recalcitrant to therapy over time. They often suffer from progressive nasal obstruction due to tenacious mucoid impaction and nasal polyps. *Aspergillus* and an abundance of eosinophils can be found in this sticky, brown mucoid material. As the fungus grows, patients can experience pain, facial swelling, and even visual disturbances in severe cases.

#### Saprophytic or chronic forms of aspergillosis

Saprophytic or chronic forms of aspergillosis of the respiratory tract develop in the setting of preexisting cavities or ectatic bronchi, such as in cavitary tuberculosis or sarcoidosis. Children with cystic fibrosis and bronchiectasis may also have saprophytic involvement of the airways due to *Aspergillus*.<sup>18</sup> Saprophytic involvement of the respiratory tract involves the development of a mass of hyphae amidst a proteinaceous matrix to form a fungus ball known as an aspergilloma.

*Aspergillus niger*, which is often the causative agent in this process, may elaborate large quantities of oxalic acid into the fungus ball and surrounding cavity. Indeed, calcium oxalate crystals may be a sign of an otherwise occult *A. niger* infection.<sup>19</sup> Local hemorrhage into the cavity may ensue, either as the result of erosion of the fungus ball into the wall of the

cavity or because of the underlying cavitory disease, such as sarcoidosis.

Aspergilloma of the respiratory tract is often a clinically occult process until the patient complains of hemoptysis. Aspergillomas also may be found during routine follow-up of patients with cavitory tuberculosis or sarcoidosis. The radiographic appearance of a rounded density within a cavity partially surrounded by a radiolucent crescent halo (Monod's sign) is characteristic of an aspergilloma. However, filamentous fungi other than *Aspergillus*, such as *P. boydii* and zygomycetes, may also cause intracavitory fungus balls and simulate an aspergilloma.

#### Invasive aspergillosis: clinical manifestations

The impact of invasive aspergillosis on patient outcome is underscored in studies by Pannuti et al<sup>10,11</sup> who found that *Aspergillus* species were the cause of 36% (20 of 55) of cases of proven nosocomial pneumonia. The crude mortality for patients with *Aspergillus* pneumonia was 95%. Further analysis indicated that elimination of 90% of cases of invasive aspergillosis would reduce the overall associated crude mortality to 43%.

Recognition of invasive pulmonary aspergillosis depends initially upon the identification of susceptible hosts. Among the patient populations at greatest risk for invasive aspergillosis are those with inadequate numbers of circulating neutrophils and those with defective neutrophil function. The most commonly infected patient populations with neoplastic diseases are those with persistent and profound granulocytopenia and/or those receiving corticosteroids.<sup>20,21</sup> Patients receiving cytotoxic chemotherapy, undergoing hematopoietic stem cell transplantation, receiving organ transplants or being treated with high-dose corticosteroids constitute a large proportion of patients with invasive pulmonary aspergillosis.

Invasive aspergillosis occurs in patients with acquired or primary defects in neutrophil function. Among patients with primary disorders of neutrophil dysfunction, those with chronic granulomatous disease and hyper-IgE (Job's) syndrome have a high predilection for recurrent episodes of invasive aspergillosis.

Patients receiving persistently high doses (>0.3 mg/kg/d) of corticosteroid therapy and patients with high endogenous levels of corticosteroids (hypercortisolemia) are at increased risk of developing aspergillosis.<sup>22</sup> Even short courses of corticosteroid therapy have been implicated in the development of invasive pulmonary aspergillosis.<sup>23,24</sup> Corticosteroids also may directly enhance the growth of *A. fumigatus*.<sup>25</sup> In a recently published 6-year survey investigating cases of invasive aspergillosis in a major medical facility, 41% of the cases of invasive aspergillosis were diagnosed in non-neutropenic patients. The most common underlying predisposing factor in these patients was the use of corticosteroids for treatment of conditions such as chronic obstructive pulmonary disease, reactive airway disease, and rheumatoid arthritis. These non-neutropenic patients tended to have less severe symptoms, but had a higher mortality rate.<sup>26</sup>

Patients with HIV are also prone to developing invasive aspergillosis. The pattern of invasive aspergillosis in HIV-infected patients is often one of extensive tracheobronchial involvement.<sup>27</sup> HIV-infected patients with predominantly large airway tracheobronchial involvement may expectorate

large mucous plugs containing hyphae. Neutropenia related to antiviral therapy, corticosteroid usage and underlying functional defects in neutrophils and monocyte-derived macrophages in HIV-infected patients may contribute to this predilection for development of pulmonary aspergillosis.<sup>28,29</sup> All of these risk factors seem to be most prevalent in patients with advanced AIDS and CD4 counts under 50/mm<sup>3</sup>.<sup>30</sup> The manifestations of invasive aspergillosis in AIDS patients are protean and can include chronic cavitory, bronchial (pseudomembranous) and invasive forms. Chronic cavitory disease may be complicated by fatal hemoptysis and high mortality.<sup>31</sup> Parenchymal invasion and dissemination in HIV-infected patients with pulmonary aspergillosis may ensue even with adequate treatment of the primary infection.<sup>30</sup>

The risk groups and clinical manifestations of invasive pulmonary aspergillosis are best understood in the context of its pathogenesis. The small 3–5 µ diameter and hydrophobic properties of *Aspergillus* conidia allow them to be carried on air currents into the alveolar air spaces. Pulmonary alveolar macrophages, which are the first line of host defense against inhaled conidia, prevent germination of conidia into hyphae. Should any conidia escape this surveillance system and germinate to form hyphae, neutrophils are capable of damaging hyphae, particularly through oxidative microbicidal pathways.

The various clinical manifestations of invasive pulmonary aspergillosis, particularly in neutropenic patients, are a reflection of the underlying pathogenesis of angioinvasion, thrombosis, and infarction. Invasive pulmonary aspergillosis in immunocompromised patients has several manifestations: pneumonia, hemoptysis, invasion of contiguous intrathoracic structures, and dissemination. These findings are not specific for aspergillosis and may be a manifestation of one of several opportunistic angioinvasive fungi (Table 27-3).

A common setting for invasive pulmonary aspergillosis is one of persistent or recurrent fever in a persistently granulocytopenic patient with pulmonary infiltrates. Patients in whom pulmonary infiltrates develop during granulocytopenia appear to have a higher risk of having pulmonary aspergillosis than those in whom pulmonary infiltrates develop during recovery from granulocytopenia.<sup>32</sup> Moreover, invasive pulmonary aspergillosis may develop in patients already receiving antifungal therapy.<sup>33</sup> Development of pulmonary infiltrates may be absent initially due to the paucity of inflammatory response; fever may be the earliest manifestation of pulmonary aspergillosis. These patients may also have pleuritic pain, non-productive cough, hemoptysis, pleural rub, and occasionally adventitious breath sounds. *Aspergillus* has a strong propensity for invasion of blood vessels, resulting in vascular thrombosis, infarction, and tissue necrosis. This process contributes to many of the clinical manifestations of pulmonary aspergillosis: pleuritic pain, pulmonary hemorrhage, hemoptysis, and cavitation.

Hemoptysis is another clinical manifestation of invasive pulmonary aspergillosis. Fungal pneumonia was found in a retrospective study to be the most common cause of fatal hemoptysis in patients with hematologic malignancies.<sup>34,35</sup> Two patterns of pulmonary hemorrhage in cancer patients were observed. The first pattern was that of hemorrhagic infarction due to vascular invasion during granulocytopenia. The second was that of the formation of mycotic aneurysms during



**Table 27-3** Patterns of invasive pulmonary infection due to angioinvasive fungi: *Aspergillus* spp., Zygomycetes, *Pseudallescheria boydii*, *Fusarium* spp.

Bronchopneumonia
Nodule
Halo sign
Crescent sign
Segmental or lobar consolidation
Cavity formation
Pleural effusion
Pulmonary vascular invasion, thrombosis, and infarction
Dissemination to extrapulmonary tissues
Invasion of chest wall, diaphragm, pericardium, and myocardium
Involvement of trachea to cause airway obstruction
Acute Pancoast's syndrome
Hemoptysis
Fistulae: <ul style="list-style-type: none"> <li>• bronchoarterial</li> <li>• bronchopleural</li> <li>• bronchocutaneous</li> </ul>
Chronic necrotizing infection*
Necrotizing tracheobronchitis*
*Best described with <i>Aspergillus</i> spp.

recovery from granulocytopenia. Neutrophils invade the walls of infected blood vessels during recovery from granulocytopenia, resulting in destruction of its elastic media in the pulmonary and bronchial blood vessels. Major vessels such as the aorta and pulmonary artery may also be involved<sup>36</sup> and occasionally occluded.<sup>37</sup> As a result of neutrophil invasion, mycotic aneurysms form and may rupture to cause potentially fatal hemoptysis in granulocytopenic patients. Thus, the new onset of hemoptysis in a persistently granulocytopenic patient, who is receiving broad-spectrum antibiotics, should prompt an investigation for the presence of *Aspergillus* spp. in the respiratory tract. Brisk hemoptysis due to pulmonary aspergillosis represents a true surgical emergency and partial lung resection can be life saving.<sup>38,39</sup>

Invasive pulmonary aspergillosis is not constrained by anatomic barriers to the parenchyma of the lung. *Aspergillus* spp. may invade through the visceral pleura to the pleural space, intercostal muscles, ribs or parietal pericardium. Once within the pericardial space, hyphal elements may invade the pericardium, causing a pericardial effusion, and continue to extend into the epicardium and myocardium, causing a myocardial infarction.<sup>40</sup>

The imaging modality of choice for early detection of invasive pulmonary aspergillosis is the high-resolution computed tomographic (CT) scan. The radiographic manifestations of invasive pulmonary aspergillosis include bronchopneumonia, nodules, halo sign, lobar consolidation, segmental pneumonia, crescent sign, and cavitary lesions.<sup>41-43</sup> Evidence of *Aspergillus* lung infection can be detected earlier in thoracic CT scans, compared to chest radiographs.<sup>44</sup>

The halo sign is a characteristic CT feature of angioinvasive organisms, including *Aspergillus* species, which should suggest invasive pulmonary aspergillosis or other mycoses.<sup>45</sup> It has been noted to be the earliest radiologic sign of pulmonary aspergillosis in neutropenic patients.<sup>46</sup> A recent retrospective study of 45 patients with invasive pulmonary aspergillosis found that small pulmonary nodules with an accompanying halo sign were present in the initial CT scans of 82% of patients.<sup>47</sup> The hazy alveolar infiltrates appear to correspond to regions of ischemia and are reversible with antifungal therapy.<sup>48</sup> Early recognition of these lesions contributes to more prompt initiation of antifungal therapy appropriate for pulmonary aspergillosis.<sup>46</sup> The halo sign, while highly specific in neutropenic patients for an angioinvasive infection such as acute invasive aspergillosis, is transient, lasting less than 5 days after the onset of the pulmonary infection in the majority of patients.<sup>46,49</sup> Other fungal organisms, including zygomycetes, *Fusarium* spp. and *Scedosporium* spp., may also produce the halo sign, as well as other radiologic features observed with aspergillosis. Earlier CT scanning in patients ultimately diagnosed with pulmonary aspergillosis resulted in earlier initiation of antifungal therapy and an improvement in survival in recent series.<sup>46,50</sup>

The crescent sign is a crescentic pulmonary cavitary lesion that is highly suggestive of the later stages of invasive pulmonary aspergillosis, generally appearing 2–3 weeks into the disease process, often in tandem with bone marrow recovery. The clinical utility of the crescent sign is thus restricted primarily to providing confirmatory evidence of the invasive *Aspergillus* infection rather than serving as a tool for early diagnosis and management of the disease.<sup>49</sup>

The appearance of new focal pulmonary infiltrates developing in the setting of granulocytopenia suggests a differential diagnosis which includes resistant bacteria (e.g., *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*), early cytomegalovirus infection, and invasive mycoses, including *Aspergillus* spp. (especially *A. fumigatus* and *A. flavus*), *Fusarium* spp., *P. boydii*, and the zygomycetes (e.g., *Rhizopus* spp., *Mucor* spp., *Cunninghamella bertholletiae*). Among patients receiving corticosteroid therapy as part of their immunosuppressive regimen or those with HIV infection, *Mycobacterium tuberculosis*, atypical mycobacteria, *Nocardia asteroides*, *Pneumocystis jiroveci*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, and *Coccidioides immitis* are included within the differential diagnosis of nodular pulmonary infiltrates due to *Aspergillus*.

Pulmonary aspergillosis in granulocytopenic and corticosteroid-treated patients often involves extrapulmonary targets. The central nervous system (CNS) is a critical target organ at risk for dissemination from the lungs and sinuses.<sup>51,52</sup> The most common manifestations of CNS aspergillosis are focal neurologic deficits, including focal seizures, hemiparesis, and cranial nerve palsies. Other target organs for disseminated aspergillosis include the eye, skin, liver, gastrointestinal tract,

kidneys, bone, and thyroid.<sup>21,53</sup> The skin may also be the portal of entry, as reported in cases of intraoperative acquisition and in those with contaminated arm boards.<sup>54-56</sup> Thus, invasive pulmonary aspergillosis in neutropenic patients should be considered as a potentially systemic infection that necessitates early intervention at the level of the sinus or pulmonary involvement to prevent extension or dissemination.

Chronic necrotizing pulmonary aspergillosis is another subset of pulmonary aspergillosis.<sup>18,57,58</sup> This indolent infection has been reported in elderly patients with chronic obstructive pulmonary disease, inactive tuberculosis, pneumoconiosis or sarcoidosis. Subtle defects in systemic host defense due to malnutrition, alcoholism, diabetes mellitus or low-dose corticosteroids may also be evident. It presents as a chronic refractory bronchopneumonia with fever, weight loss, cough, progressive infiltrates, and evidence of invasive aspergillosis on biopsy. This infection may progress to cavitation and formation of an aspergilloma or may develop from an aspergilloma as the initial focus. Alternatively, an aspergilloma in such patients may progress to invade the surrounding pulmonary parenchyma. The course of this infection may evolve over months unless antifungal therapy is initiated.

*Aspergillus* sinusitis is a highly invasive process that may develop before or concomitantly with pulmonary aspergillosis in immunocompromised patients.<sup>59-61</sup> The infection may spread to the orbit, resulting in proptosis, chemosis, and cutaneous necrosis. Direct extension from the orbit can cause frontal lobe infection, which has the potential to lead to cavernous sinus thrombosis. In contrast to the serious nature of *Aspergillus* infections, patients may have a paucity of symptoms in the early stages of the disease. Initial complaints may only consist of mild rhinorrhea or congestion and patients often do not have significant nasal discharge or sinus tenderness. These symptoms should not be dismissed as viral rhinitis in an immunocompromised host.

Prompt recognition of the initial stages of *Aspergillus* sinusitis through a comprehensive physical examination, early otolaryngology referral, and radiographic imaging can enable the clinician to commence appropriate antifungal therapy early; this approach can obviate the need for invasive sinus drainage procedures and mitigate the extent of the disease process. A careful nasal speculum exam may reveal sentinel eschars along the mucosa of the nasal turbinates. Erythema along one-half of the palatal mucosa ipsilateral to the infected sinus may also be evident. Radiographs and CT scans of the sinuses often demonstrate air-fluid levels or complete opacification. Bony destruction, retroorbital infiltration, and CNS infection also may be evident in more advanced stages. Biopsy and culture of mucosal lesions may reveal invasive aspergillosis. If nasal septal lesions are not observed, a sinus aspirate may preclude the need for bronchoscopy if fungus is demonstrated in the aspirate. Although *Aspergillus* spp. are the most common cause of fungal sinusitis in immunocompromised patients, other fungi including zygomycetes, *Fusarium* spp., *P. boydii*, *Curvularia* spp., and *Alternaria* spp. may be isolated.

#### Invasive aspergillosis: microbiologic diagnosis

The most common species of *Aspergillus* recovered from patients are *A. fumigatus*, *A. flavus*, and *A. niger*. While less frequently isolated, *A. terreus*, *A. ustus*, and *A. nidulans* are also known pulmonary pathogens in humans. While *A. fumigatus*

continues to be the most common cause of invasive aspergillosis, reports of infections due to other *Aspergillus* species in cancer patients have been increasing in frequency. A recently published retrospective analysis of 40 cases of invasive aspergillosis in a tertiary care cancer center reported that 70% of cases were due to non-*fumigatus* species. *A. flavus* and *A. terreus* accounted for 46% and 39% of the non-*fumigatus* infections, respectively. In this study, *A. fumigatus* was more likely to be the causative agent in late-onset aspergillosis following stem cell transplantation.<sup>62</sup> *Aspergillus niger* is commonly isolated in saprophytic conditions, such as chronic obstructive pulmonary disease and chronic sinusitis, but is seldom proven to be a cause of invasive pulmonary aspergillosis in immunocompromised patients.

The significance of recovery of *Aspergillus* spp. from clinical specimens must be underscored. *Aspergillus* spp., particularly *A. fumigatus*, are uncommon contaminants in most clinical microbiology laboratories. *Penicillium* spp. in our experience have been considerably more frequent as a laboratory contaminant. One should therefore carefully evaluate the significance of *Aspergillus* spp. isolated from a diagnostic specimen.

*Aspergillus* spp. in tissue form hyaline angular dichotomously branching septate hyphae. The invasive tissue form has no conidiophores, vesicles, phialides or conidia. These structures may occasionally be seen, however, in cavitary lesions that communicate directly with the tracheobronchial tree. The organism in tissue is usually distinguishable from *Candida* spp., which has pseudohyphae and blastoconidia. However, when *Aspergillus* hyphae in tissue are sectioned in cross-section they may resemble non-budding yeast forms. The histopathologic pattern of angular, dichotomously branching septate hyphae may be observed in invasive tissue infection due to *Aspergillus* spp., *Pseudallescheria boydii*, *Fusarium* spp., and several less common fungi. An example of confusion between *Aspergillus* and *P. boydii* is highlighted in a recent case report.<sup>63</sup> *Pseudallescheria boydii* may occasionally be observed in tissue to develop terminal conidia. Nevertheless, a culture diagnosis is the only way to distinguish these invasive fungi. Biopsy and culture of tissue is the most definitive means by which to establish a diagnosis of invasive aspergillosis. However, since many patients at risk for invasive aspergillosis also have hemostatic defects which preclude invasive diagnostic procedures, alternative approaches to establish a presumptive diagnosis are often initially pursued.

Isolation of *Aspergillus* spp. from respiratory tract cultures of febrile granulocytopenic patients with pulmonary infiltrates should be considered a priori evidence of pulmonary aspergillosis. In a prospective study, Yu et al<sup>64</sup> found that isolation of *Aspergillus* spp. from respiratory secretions of high-risk patients was highly predictive of invasive pulmonary aspergillosis. Among 108 consecutive patients from whom *Aspergillus* spp. were isolated, 17 patients with granulocytopenia and/or leukemia had lung tissue examined; all had invasive pulmonary aspergillosis. Invasive aspergillosis was not found in non-immunosuppressed patients or in non-granulocytopenic patients with solid tumors. Multivariate analysis demonstrated that granulocytopenia and absence of smoking were the most significant predictors of invasive aspergillosis in patients with respiratory tract cultures growing *Aspergillus* spp. The findings of Treger et al<sup>65</sup> in a retrospective study also underscored the significance of isolation of *Aspergillus* spp. from respiratory

secretions in high-risk populations. *Aspergillus* spp. were rarely contaminants in respiratory secretions. In contrast to granulocytopenic patients, Yu et al<sup>64</sup> found a low predictive value for invasive disease when *Aspergillus* spp. were recovered from respiratory secretions of non-granulocytopenic smokers with chronic lung disease.

The presence of *Aspergillus* spp. in bronchoalveolar lavage (BAL) fluid of febrile neutropenic patients with new pulmonary infiltrates is indicative of invasive aspergillosis; however, the absence of hyphal elements or positive culture does not exclude the diagnosis.<sup>66,67</sup> Even the presence of hyphae on direct examination of culture-negative BAL in a febrile neutropenic patient with progressive pulmonary infiltrates refractory to antibiotics should be considered a priori evidence of invasive pulmonary aspergillosis. Bronchoscopy and high-resolution CT scans are complementary diagnostic tools and should be performed as early as possible in the course of pneumonia for patients at risk of invasive pulmonary aspergillosis.

Peripheral nodules may be more readily accessible by CT-guided percutaneous needle aspirate. Recent usage of thoracoscopy may allow high-risk patients with peripheral lesions to be diagnosed with certainty. If the foregoing methods do not yield a microbiologic diagnosis or are not feasible, then video-assisted thoracoscopy (VAT) or open lung biopsy (OLB) may be performed. For patients with a localized infiltrate, however, OLB will require a thoracotomy using either a lateral or mediastinal approach. It is imperative that the surgeon obtains the biopsy of both the periphery as well as the central areas of abnormal lung, since the distribution of organism may vary. The presence of a positive serum galactomannan may preclude the need for OLB.

Important advances are being achieved in the immunodiagnostic and molecular methodology for early detection of invasive pulmonary aspergillosis.<sup>68</sup> In the 1970s and 1980s, galactomannan, a polysaccharide cell wall component found in most *Aspergillus* species, was found to be circulating in serum and present in urine in experimental disseminated aspergillosis.<sup>69,70</sup> Measurement of galactomannan levels in serum and BAL fluid is being increasingly utilized in the early diagnosis of aspergillosis as well as to monitor treatment efficacy. The commercial assay most commonly used in Europe and the USA is the Platelia ELISA (Biorad, Marnes-La-Coquette, France). The reported sensitivity of the assay has varied widely, with a range of 29–100%.<sup>68,71</sup> The reasons for this variance appear to be multifactorial. The sensitivity of galactomannan is highest in severely immunocompromised patients such as neutropenic patients with hematologic malignancies and HSCT recipients, and has been noted to be significantly lower in host populations with stronger immune defenses (e.g., recipients of solid organ transplants).<sup>72–77</sup> Several studies have also demonstrated impaired galactomannan sensitivity in patients receiving antifungal therapy.<sup>78–80</sup> In addition, variability in the timing and frequency of galactomannan screening may lead to suboptimal sensitivity in some cases.<sup>68</sup> In general, a twice-weekly galactomannan measurement has been judged to be an adequate screening approach for asymptomatic high-risk patient populations. Of course, galactomannan measurements should be obtained as soon as possible in patients who have a clinical picture compatible with invasive aspergillosis.

In contrast to the variation in sensitivity, galactomannan specificity has been consistently estimated to be above

90%.<sup>75,76,78,79,81–83</sup> False-positive results have been noted in patients receiving  $\beta$ -lactam antibiotics, especially piperacillin/tazobactam.<sup>84,85</sup> Although galactomannan specificity was thought to be lower in children and neonates, more recent studies indicate that galactomannan in pediatric oncology patients has similar specificity to that of adults.

Real-time and nested polymerase chain reaction (PCR)-based methodologies have also been demonstrated to be potentially valuable tools in the early detection of *Aspergillus* infection. The numerous studies performed to date assessing the efficacy of PCR reflect the promise of the technique, but also demonstrate variability in its sensitivity and specificity as well as the lack of a standardized platform.<sup>86</sup> Some studies have reported comparable or superior sensitivity and specificity of PCR compared to *Aspergillus* galactomannan,<sup>87,88</sup> while others have shown PCR to be inferior.<sup>89–91</sup> Further studies are necessary to provide a consensus regarding standardization, optimal timing and selection of sample sources (BAL, serum, etc.), as well as to clarify the patient populations for whom PCR screening would be most beneficial.

Bronchoalveolar lavage is a cornerstone in the early detection of pulmonary *Aspergillus* infection. One primary benefit is that it is a relatively non-invasive, straightforward procedure that is well tolerated by most patients. Unfortunately, its diagnostic yield by traditional culture-based methodology has been historically low. In addition, a positive *Aspergillus* culture can require days to be detected. Galactomannan antigen detection and real-time PCR assays of BAL washings have been recently studied as adjunctive tests for the early detection of invasive aspergillosis. A recent study comparing these methodologies in a rabbit animal model of experimentally induced *A. fumigatus* confirmed the relatively low sensitivity and high specificity of quantitative culture methods (46% and 100%, respectively). In contrast, galactomannan EIA and quantitative PCR assays demonstrated higher sensitivities, with galactomannan antigen detection being superior to PCR (100% and 80% sensitivity, respectively).<sup>92</sup> These findings are consistent with those of studies of galactomannan in BAL fluid in patients with invasive pulmonary aspergillosis.<sup>93</sup>

Another method of detection and monitoring *Aspergillus* infections is via serum measurements of (1,3)- $\beta$ -D-glucan (GlucateLL, Cape Cod Associates). This colorimetric assay is now commercially available in the United States to aid in the diagnosis of invasive fungal infections. (1,3)- $\beta$ -D-glucan is a prominent cell wall component of most fungi, with the exception of the zygomycetes and *C. neoformans*. As a result, it has more utility as a marker for the presence and extent of an invasive fungal infection rather than in establishing a specific diagnosis of aspergillosis. Its potential as a surrogate marker for infection was highlighted in a recent retrospective analysis comparing the efficacy of (1,3)- $\beta$ -D-glucan assays with galactomannan screening of 40 neutropenic adult patients at risk of invasive aspergillosis. In this study, the sensitivity and specificity of both tests were identical, measured at 87.5% and 89.6% respectively. Both tests signaled the presence of infection prior to CT abnormalities and clinical symptoms in most patients.<sup>94</sup> A recent prospective comparison of serum galactomannan ELISA with real-time PCR and (1,3)- $\beta$ -D-glucan tests in 96 hematology patient demonstrated superior sensitivity of two serial ELISA measurements (1.00 versus 0.55 for both PCR and (1,3)- $\beta$ -D-glucan) but similar specificities in the



93% range.<sup>95</sup> The potential of (1,3)- $\beta$ -D-glucan as a surrogate marker of infection was highlighted in a recent study in which serum levels were elevated in seven out of eight patients with invasive pulmonary aspergillosis. These results, however, are preliminary and this assay remains investigational.<sup>96</sup>

In conclusion, the timely diagnosis of invasive aspergillosis remains challenging due to the lack of specific and significant symptoms early in the disease in most immunocompromised patients, as well as the need for consensus regarding the optimal screening strategy. Today's clinician has an expanding repertoire of tools to aid in the timely diagnosis of invasive aspergillosis, including traditional histologic and culture-based methods, radiologic screening, and newer ELISA, PCR and colorimetric methodologies. Further study is warranted to determine the timing and combination of tests that will most consistently, accurately and rapidly detect *Aspergillus* infections.

## Treatment of aspergillosis of the respiratory tract

### Allergic aspergillosis

The current treatment of ABPA consists of corticosteroids (e.g., prednisone) and itraconazole (Table 27-4). Early aggressive therapy may attenuate progression to an irreversible fibrotic phase. Chronic *Aspergillus* sinusitis in immunocompetent adults requires surgical drainage. Itraconazole has been shown to be effective in combination with oral corticosteroids, topical corticosteroids and surgery for the management of allergic fungal sinusitis.<sup>97</sup>

### Aspergilloma

Treatment of aspergilloma is individualized according to the severity of symptoms and the underlying chronic lung disease. Current therapeutic approaches include conservative management and surgical resection. Patients with chronic necrotizing pulmonary aspergillosis are managed with voriconazole or itraconazole (see Table 27-4).

Severe underlying chronic lung disease may limit surgical resection of aspergillomas. Recurrent or life-threatening hemoptysis despite antifungal chemotherapy is a relative indication for surgical intervention. Data suggest that lung resection (segmentectomy, lobectomy, completion pneumonectomy) in selected patients with invasive aspergilloma can be performed with low operative mortality.<sup>98,99</sup> Overall outcome is dependent upon the severity of the patient's pulmonary and co-morbid conditions, delays in diagnosis and initiation of effective therapy.<sup>58</sup>

### Acute invasive aspergillosis

Successful antifungal therapy of invasive pulmonary aspergillosis in immunocompromised patients depends upon early initiation of antifungal medication and reversal of immunosuppression. Early diagnosis and prompt administration of appropriate antifungal therapy have been well recognized as critical factors in survival of patients with invasive aspergillosis.<sup>100,101</sup> Recovery from granulocytopenia, reduction of corticosteroid therapy, and amelioration of other potential immunosuppressive factors are also critical factors in successful treatment of invasive pulmonary aspergillosis in cancer patients.

Voriconazole is used as primary therapy for invasive aspergillosis in lieu of conventional amphotericin B (see Table 27-4). A large multinational collaborative randomized comparative study conducted from 1997 to 2000 clearly demonstrated the advantages of voriconazole over conventional amphotericin B. After 12 weeks of therapy, complete or partial responses were achieved in 52.8% of patients treated with voriconazole compared to 31.6% of those treated with amphotericin B deoxycholate. Furthermore, 70.8% of patients who received voriconazole survived, compared to 57.9% of patients who were assigned to the amphotericin B group. In addition, patients receiving voriconazole experienced fewer treatment-related side effects.<sup>102</sup> Dosing of voriconazole is 6 mg/kg twice daily on day 1, followed by 4 mg/kg twice daily intravenously for at least 7 days in adults. When patients are deemed stable enough, they may then switch to oral voriconazole (200 mg twice daily) to complete an average of 12 weeks of therapy.

Voriconazole lacks the nephrotoxicity and infusion-related electrolyte imbalances associated with amphotericin B. Hepatotoxicity, transient visual disturbances and visual hallucinations have been associated with voriconazole.<sup>102</sup> Clinicians prescribing voriconazole should diligently check for possible drug interactions, since voriconazole can interact adversely with or alter the metabolism of several commonly used medications. Notably, it is recommended that cyclosporine dosages be reduced by 50% in patients who are commencing voriconazole.

Cornely et al conducted a randomized trial comparing two dosages of liposomal amphotericin B in the treatment of invasive aspergillosis. Similar efficacy was demonstrated in both the low dose (3 mg/kg/day) and higher dose (10 mg/kg/day) groups; the overall response rate in patients with invasive aspergillosis who received the 3 mg/kg/day dosage regimen was 50%. These data suggest that liposomal amphotericin B could be considered as alternative primary therapy in patients with invasive pulmonary aspergillosis for whom voriconazole is contraindicated.<sup>103</sup>

Defining standard optimal salvage therapy for invasive aspergillosis that is refractory to voriconazole remains challenging due to a lack of comprehensive clinical data. The optimal regimen should take into account individual host factors such as co-existing medical problems and medication profiles. Alternative salvage regimens include changing the class of antifungal agent from a triazole to a lipid amphotericin B formulation, or to an echinocandin such as caspofungin or micafungin. In a host with underlying renal insufficiency, the addition of an echinocandin to voriconazole, or switch to posaconazole or an echinocandin may be beneficial. Conversely, a patient with hepatic disease may benefit from the combination of an echinocandin with a lipid formulation of amphotericin B or from a single-agent antifungal regimen of an echinocandin or lipid formulation of amphotericin B alone.<sup>104</sup>

Combination therapy with echinocandin and triazole antifungal agents has been demonstrated to be synergistic in vitro and in vivo against *A. fumigatus*. Observational or retrospective clinical studies are consistent with this enhanced efficacy.<sup>105</sup> However, whether the combination of voriconazole and an echinocandin is more active than voriconazole alone as primary therapy for invasive aspergillosis is not known. Not

**Table 27-4** Summary of approaches to treatment of fungal infections of the respiratory tract

<b>Allergic aspergillosis</b>	
<ul style="list-style-type: none"> <li>• Extrinsic allergic alveolitis</li> <li>• Extrinsic asthma</li> <li>• Allergic bronchopulmonary aspergillosis (ABPA)</li> </ul>	Removal of patients from exposure to antigen Bronchodilators Corticosteroids Itraconazole (ABPA) Voriconazole
<b>Saprophytic aspergillosis</b>	
Pulmonary aspergilloma	Observation Surgical resection (usually indicated for intractable hemoptysis and pain) Itraconazole (doubtful benefit) Voriconazole (doubtful benefit)
<b>Invasive aspergillosis</b>	
Bronchopneumonia Necrotizing tracheobronchitis Invasive sinusitis Chronic necrotizing aspergillosis Local extension to intrathoracic structures Disseminated aspergillosis	Voriconazole Lipid formulation of amphotericin B Deoxycholate amphotericin B Itraconazole (salvage) Posaconazole (salvage) Echinocandin (salvage) Combination antifungal therapy? Reversal of immunosuppression (refer to Table 27-5) Surgery: (1) hemoptysis from a single cavitary lesion (2) progression of a cavitary lesion despite antifungal therapy (3) infiltration into pericardium, great vessels, bone or thoracic soft tissue while receiving antifungal therapy (4) progressive sinusitis
<b>Zygomycosis</b>	
Rhinocerebral Pulmonary	Lipid formulation of amphotericin B Amphotericin B Posaconazole (second-line therapy) Possible combination therapy with echinocandin Surgical debridement of rhinocerebral infection to viable tissue Surgery for pulmonary infection: refer to invasive aspergillosis Reversal of immunosuppression Correction of metabolic acidosis or hyperglycemia Removal of deferoxamine Immune augmentation (GCSF or GM-CSF) Possible administration of deferasirox
<b><i>Pseudallescheria boydii</i> (<i>Scedosporium apiospermum</i>)</b>	
Pulmonary infection	Voriconazole Itraconazole Antifungal azole plus amphotericin B Surgery for pulmonary infection: refer to invasive aspergillosis Reversal of immunosuppression

**Table 27-4** Summary of approaches to treatment of fungal infections of the respiratory tract—cont'd

<b><i>Fusarium</i> infection</b>	Voriconazole Lipid formulation of amphotericin B Amphotericin B Posaconazole Reversal of immunosuppression
<b>Pulmonary histoplasmosis</b>	Observation in selected normal hosts Itraconazole Lipid formulation of amphotericin B Amphotericin B Reversal of immunosuppression
<b>Pulmonary coccidioidomycosis</b>	Observation in selected normal hosts Itraconazole Fluconazole Voriconazole Ketoconazole Amphotericin B Reversal of immunosuppression
<b>Pulmonary blastomycosis</b>	Itraconazole Voriconazole Amphotericin B
<b>Pulmonary paracoccidioidomycosis</b>	Itraconazole Ketoconazole Amphotericin B Trimethoprim-sulfamethoxazole
<b>Penicilliosis</b>	Itraconazole Amphotericin B
<b>Pulmonary sporotrichosis</b>	Itraconazole Amphotericin B
<b>Pulmonary cryptococcosis</b>	Amphotericin B +/- flucytosine Fluconazole Itraconazole
<b>Pulmonary candidiasis</b>	Echinocandin Fluconazole (8–10 mg/kg/day) Lipid formulation of amphotericin B Amphotericin B Reversal of immunosuppression
<b><i>Trichosporon</i> infection</b>	Fluconazole Voriconazole Reversal of immunosuppression Recombinant cytokines (GM-CSF or interferon- $\gamma$ )

all combinations of antifungal compounds are beneficial; for example, amphotericin B and triazoles can be antagonistic in vitro and in vivo.<sup>106</sup> Recovery from granulocytopenia, reduction of corticosteroid therapy, and amelioration of other potential immunosuppressive factors are critical factors in successful treatment of invasive aspergillosis in immunocompromised hosts. Unless immunosuppression is reversed or substantially

ameliorated, the prognosis of opportunistic invasive aspergillosis is dismal.

Surgical resection in invasive pulmonary aspergillosis may be employed in several specific conditions:

- hemoptysis from a single cavitory lesion
- progression of a cavitory lesion despite antifungal therapy



- infiltration into bone or thoracic soft tissue while receiving antifungal therapy
- progression of infection in a critical target organ, such as the central nervous system or pericardium.

Early resection combined with appropriate antifungal therapy has been used in an aggressive approach for localized infection in selected patients.<sup>46,107,108</sup> Of course, the approach to treatment must be individualized for each patient.

Control of environmental transmission of conidia can be an important adjunct in managing an outbreak of nosocomial aspergillosis. The capacity of *A. fumigatus* to establish a pulmonary infection in an immunocompromised host depends upon the level of inoculum.<sup>109,110</sup> Consistent with these findings are the observations that large pulses of conidia from contaminated environmental sources appear to present a particularly high risk for development of pulmonary aspergillosis in immunocompromised patients.<sup>111</sup> A review of nosocomial invasive aspergillosis found that the most common environmental sources of *Aspergillus* in hospital outbreaks were contaminated air-conditioning units and construction sites.<sup>6</sup> Another study demonstrated microbiologic evidence that endemic and epidemic aspergillosis are associated with in-hospital replication of *Aspergillus* organisms.<sup>112</sup> Following removal of the contaminated air filters and the environmental foci, there was a greater than 100-fold reduction in *Aspergillus* counts and a fourfold decrease in invasive aspergillosis during the subsequent 2 years of the study. Floor-to-ceiling barriers for prevention of transmission of *Aspergillus* conidia to high-risk populations should be established in hospital areas of construction or renovation. Air-conditioning systems should be microbiologically monitored, especially during periods of repair or malfunction. High-efficiency particulate air (HEPA) filters should be utilized, when possible, in hospital areas with patients having profound protracted granulocytopenia (e.g., allogeneic HSCT recipients). Reports from Anaissie et al suggest that hospital water systems may be a potential source of aerosolization of *Aspergillus* and *Fusarium* spp., but further studies are needed.<sup>113,114</sup> Appropriate environmental and infection control measures should be implemented in cooperation with hospital infection control authorities, hospital engineering staff, and physicians caring for immunocompromised patients when air-conditioning repairs or construction are performed within a medical facility.

Strategies for prevention of invasive fungal infection have been intensively studied in neutropenic patients.<sup>115-117</sup> There are three complementary preventive sequential strategies in neutropenic hosts: prophylaxis, empiric, and preemptive therapy. Prophylactic interventions begin during or soon after completion of cytotoxic chemotherapy. Empiric antifungal therapy is initiated in patients who remain persistently neutropenic and febrile despite broad-spectrum antibiotics, and preemptive therapy is initiated following antigenic and radiologic evidence of infection. We will confine our discussion to prophylaxis. Itraconazole, which is erratically absorbed from the gastrointestinal tract, may not achieve adequate plasma levels to prevent the development of aspergillosis.<sup>118</sup> Moreover, gastrointestinal intolerance of the oral cyclodextrin formulation may limit its utility. The parenteral formulation of itraconazole may overcome this pharmacokinetic limitation. A recent metaanalysis of prophylactic itraconazole

demonstrated a reduction of risk of invasive fungal infections by 42%.<sup>119</sup>

Posaconazole was recently licensed for prophylaxis against invasive aspergillosis and other mycoses. Supporting the use of posaconazole for prophylaxis are two recently published prospective studies demonstrating that it was superior to itraconazole and fluconazole in the prevention of invasive aspergillosis in high-risk neutropenic patients undergoing chemotherapy or on immunosuppressive medications for graft-versus-host disease (GVHD) prophylaxis.<sup>120-122</sup> In addition, a small double-blind comparison of prophylactic oral voriconazole with placebo in the prevention of invasive fungal infections in patients undergoing induction chemotherapy for acute myeloid leukemia (AML) revealed a marked reduction in pulmonary infiltrates in the group on voriconazole prophylaxis.<sup>123</sup>

Secondary prophylaxis is yet another strategy for prevention of invasive aspergillosis in oncology patients and HSCT recipients. Most patients with leukemia, lymphoma, and various solid tumors undergo several cycles of intensive chemotherapy, and thus have predictable windows of time when they may be especially vulnerable to fungal infections. If pulmonary aspergillosis develops in neutropenic patients who will require subsequent cytotoxic chemotherapy, the risk of recurrence of pulmonary aspergillosis is approximately 50%.<sup>64</sup> Thus, one approach to managing such patients with a history of invasive aspergillosis is to administer voriconazole at the earliest onset of fever and granulocytopenia. This approach to early initiation of voriconazole therapy may be considered a form of secondary prophylaxis.<sup>124</sup> Treatment of the underlying neoplastic process is essential for survival. Cytotoxic chemotherapy may be continued in cancer patients with an invasive mycosis, provided that the fungal infection is controlled.<sup>125-127</sup> While there is more experience with chronic disseminated candidiasis than with aspergillosis in this setting, the same principles are applicable.

Protracted neutropenia is one of the major risk factors for development of invasive pulmonary aspergillosis in patients with neoplastic diseases. The recombinant human cytokines, such as granulocyte colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF), can shorten the duration of granulocytopenia, activate pulmonary alveolar macrophages, and help mitigate the risk of developing invasive aspergillosis in oncology patients and HSCT recipients (Table 27-5).<sup>128,129</sup> A shorter duration of granulocytopenia due to cytokine therapy in acute myelogenous leukemia has been shown to decrease the incidence of infection in some<sup>130</sup> but not all<sup>131</sup> studies and may decrease the frequency of invasive aspergillosis. Earlier recovery from granulocytopenia may also facilitate resolution of established *Aspergillus* lesions in conjunction with effective antifungal therapy. Neutrophil transfusions from G-CSF stimulated donors (5 mg/kg/day SC) have been administered as adjuncts to antifungal chemotherapy in the treatment of established fungal infections.<sup>130,132</sup>

## Zygomycosis

Zygomycosis is an increasingly reported and often fatal group of infections caused by members of the class Zygomycetes. The spectrum of zygomycosis includes rhinocerebral infections

**Table 27-5** Reversal of immunosuppression: immunologic adjuncts to prevention and treatment of pulmonary fungal infections

Recombinant cytokines
granulocyte colony stimulating factor (GCSF)
granulocyte-macrophage colony stimulating factor (GMCSF)
interferon- $\gamma$
macrophage colony stimulating factor (MCSF)
Stem cell reconstitution
Immune reconstitution
Granulocyte transfusions
Adaptive immunotherapy
Discontinuation of corticosteroids

complicating diabetes mellitus, pulmonary or disseminated infections emerging during granulocytopenia or corticosteroid therapy, and disseminated infection developing during deferoxamine therapy (Table 27-6). A recent comprehensive epidemiologic analysis of 929 reported cases highlighted the diversity of patients affected by zygomycetes. In contrast to other filamentous fungal infections that primarily infect classically immunocompromised hosts, over 50% of patients with zygomycosis had diabetes or no identifiable predisposing condition. One half of those patients without an underlying disease presented with cutaneous involvement. In this study, a clear relationship between pulmonary zygomycosis and malignancy and HSCT was demonstrated. This may be due to chemotherapy-induced damage to innate pulmonary host defenses and mucociliary clearance mechanisms.<sup>133</sup> Although pulmonary zygomycosis is most frequently observed in granulocytopenic and corticosteroid-treated patients,<sup>134-143</sup> those with HIV infection are also at risk.<sup>144-146</sup> Rhinocerebral zygomycosis occurs predominantly in patients with uncontrolled diabetic ketoacidosis and in patients with pharmacologically induced immunosuppression, such as corticosteroid therapy or cytotoxic chemotherapy-mediated granulocytopenia. Patients with renal failure, diabetes mellitus, and those receiving deferoxamine therapy also have a predisposition for development of rhinocerebral zygomycosis.<sup>147-149</sup> Most of the zygomycetes causing respiratory infections in immunocompromised or debilitated hosts have a high propensity for thrombotic invasion of blood vessels, for causing a rapidly evolving clinical course, high mortality, and a relative resistance to antifungal therapy.<sup>150-152</sup>

The class Zygomycetes is composed of medically important orders: the Mucorales and the Entomophthorales.<sup>153,154</sup> Respiratory infections are usually caused by fungi of the order Mucorales. The most frequently encountered agent is *Rhizopus oryzae*. Among the order Mucorales, other species, such as *Cunninghamella bertholletiae*<sup>153,155-157</sup> and *Absidia corymbifera*, have been increasingly reported as respiratory pathogens. Members of the Entomophthorales typically cause tropical subcutaneous zygomycosis (lobomycosis) and another

**Table 27-6** Risk factors associated with development of respiratory zygomycosis

Diabetic ketoacidosis
Other forms of chronic metabolic acidosis (rare)
Diabetes mellitus
Corticosteroid therapy
Granulocytopenia
Iron overload states
Deferoxamine therapy
Burns or trauma
Low birth weight or neonatal prematurity

form of zygomycosis affecting the nasal submucosa (rhinotomophthoromycosis); they rarely cause pulmonary and disseminated zygomycosis.<sup>158</sup>

In studies of *R. oryzae*, asexual sporangioconidia (measuring 5–8  $\mu$ ) are inhaled into the distal airways, where they may be cleared by pulmonary alveolar macrophages (PAMs), the main source of host defense against sporangioconidia.<sup>159-161</sup> If PAMs are impaired by corticosteroids or other immunosuppressive agents, they may fail to clear the sporangioconidia, permitting germination and development of hyphae. Polymorphonuclear leukocytes (PMNs) are the main host defense against zygomycetous hyphae, such that hyphae may progress relentlessly in neutropenic hosts.

Angioinvasion due to zygomycetes may result in infarction with potentially lethal intrapulmonary hemorrhage as the result of concomitant thrombocytopenia. The extent of tissue infarction in the distribution of an occluded blood vessel often extends beyond the region of infected tissue. This propensity for angioinvasion may become clinically evident as pulmonary infarctions, pulmonary artery aneurysms, and hemorrhage.<sup>139,140,161-164</sup> This same process of thrombosis and infarction occurs in other sites, accounting for clinical manifestations of zygomycosis in these tissues.<sup>165-171</sup> Diabetic ketoacidosis and other forms of chronic metabolic acidosis can impair innate host defenses.<sup>148,159-161,172-179</sup>

The importance of iron availability in host–fungus interaction in zygomycosis is underscored by the important observations of disseminated zygomycosis developing in patients receiving iron chelation therapy with deferoxamine.<sup>180-191</sup>

## Clinical manifestations of respiratory zygomycosis

As rhinocerebral and pulmonary zygomycosis are among the most fulminant fungal infections, early recognition and intervention are critical for a successful outcome. Rhinocerebral zygomycosis usually begins as an infection of the maxillary and ethmoid sinuses, which progresses to invade the orbit, retroorbital region, cavernous sinus, and brain.<sup>139,192-194</sup>

A black eschar on the palatine or nasal mucosa and blackened discharge from the eye are clinical manifestations of infarction. Black, necrotic lesions on the palate or nasal mucous membranes may also be caused by other fungi, including *Aspergillus* spp., *Fusarium* spp., and *P. boydii*.

Initial symptoms of rhinocerebral zygomycosis include unilateral headache, ocular irritation, chemosis, lacrimation, periorbital swelling, blurred vision, periorbital numbness, nasal congestion, and epistaxis.<sup>139,140,150</sup> The complaint of diplopia or new onset of blurred vision from a diabetic patient, a patient receiving deferoxamine or a pharmacologically immunosuppressed patient should prompt a careful examination for early rhinocerebral zygomycosis.<sup>195</sup> Orbital or facial cellulitis or proptosis occurs in approximately two-thirds of cases of rhinocerebral zygomycosis.<sup>196</sup> Black, necrotic lesions may be found on the hard palate or on the nasal mucous membranes. Infection of the palate may extend into the paranasal sinuses. Involvement of the ethmoid sinuses may be complicated by cavernous sinus thrombosis. Early neurologic manifestations of cavernous sinus thrombosis include paralysis of the second, third, fourth, and sixth cranial nerves as well as the first and second divisions of the fifth nerve, resulting in loss of vision, internal and external ophthalmoplegia, corneal anesthesia and facial anhidrosis. As more advanced stages of this infection carry a dismal prognosis, early recognition is important.

Emergent use of CT scans or magnetic resonance imaging (MRI) defines extent of infection and guides surgical resection of infected tissue.<sup>197</sup> Radiographic manifestations of rhinocerebral zygomycosis include fluid in or clouding of the paranasal sinuses, bone destruction or osteomyelitis.<sup>197-205</sup>

Pulmonary zygomycosis occurs especially in patients with profound neutropenia or corticosteroid therapy, as well as in patients with renal transplantation, iron chelation therapy, autoimmune diseases treated with corticosteroid therapy, and HIV infection.<sup>134,139-142,198,206</sup> Pulmonary zygomycosis in granulocytopenic patients resembles pulmonary aspergillosis, with persistent fever and pulmonary infiltrates refractory to antibacterial therapy.<sup>21</sup> The clinical manifestations of pulmonary zygomycosis reflect its pathophysiology. Radiographically, consolidation involving one or multiple lobes, nodules, masses, cavitation, and pleural effusions may be seen.<sup>207,208</sup> Potentially fatal hemoptysis may develop in thrombocytopenic patients<sup>35,163,209</sup> and occasionally in those who have attained a complete hematologic remission in a manner reminiscent of invasive aspergillosis.<sup>210</sup> Other manifestations include endobronchial masses, erosion of bronchi, bronchopleural and bronchocutaneous fistulae, and zygomycetous granulomatous mediastinitis.<sup>164,211-214</sup> The infection may also invade directly across tissue planes to involve the chest wall, diaphragm, pericardium, and myocardium.

Several patients with a subacute pulmonary zygomycosis have been reported.<sup>215</sup> A rare case of indolent zygomycosis due to *Rhizopus* spp. associated with deferoxamine therapy has been reported.<sup>216-218</sup> The illness may smolder for weeks to months.

## Diagnosis of zygomycosis

As proven effective therapeutic modalities are limited to conventional and lipid formulations of amphotericin B, pulmonary zygomycosis warrants a high degree of suspicion and an

aggressive approach toward diagnosis.<sup>219,220</sup> Examination by Calcofluor staining or by KOH-digested sputum and cultures of respiratory tract secretions are frequently negative. Computed tomography can define the extent of disease and can guide fine needle aspiration or thoracoscopy.

Examination of wet mounts of sputum and cultures of respiratory tract secretions is frequently negative. Recovery of a zygomycete from a BAL specimen in an immunocompromised patient with fever and pulmonary infiltrate should not be dismissed as a contaminant;<sup>221</sup> such an isolate in the appropriate setting may represent strong evidence of invasive pulmonary zygomycosis. If sputum examination and evaluation of BAL specimens are non-diagnostic, more invasive diagnostic tests may be warranted. Fine needle aspirate, thorascopic or open lung biopsy may be considered, depending on local expertise and pace of the illness. Suspicious cutaneous lesions should also be biopsied.

Organisms in tissue exhibit broad (15–20  $\mu$ ), irregular, usually sparsely septate (coenocytic) hyphae with non-dichotomous side branching. Swab cultures of infected sinuses may be negative. Chandler et al reported the formation of chlamydoconidia in tissue in four cases of zygomycosis due to either *Absidia* or *Rhizopus* spp.<sup>222</sup> A negative culture result from tissue does not exclude or even diminish the probability of zygomycosis. Tissue specimens should be stained promptly with Gomori methenamine silver nitrate and with hematoxylin and eosin. In addition, a portion should be examined on a slide with 20% aqueous potassium hydroxide with Calcofluor under a fluorescent microscope. Tissue specimens for culture are inoculated onto appropriate media, for example Sabouraud dextrose agar and potato dextrose agar (containing no added cycloheximide), and incubated at room temperature and at 37°C.<sup>223</sup> Zygomycetes may be rendered non-viable if infected tissue is ground or homogenized in preparation for plating on culture media. The recovery rate may be enhanced if the tissue specimen is sliced into small pieces without grinding or homogenization.

## Treatment of respiratory zygomycosis

Sinus drainage, debridement of infected tissue, and intravenous amphotericin B are the cornerstones of therapy in rhinocerebral zygomycosis.<sup>134,139,224-229</sup> Surgical excision of infected lesions is also important in the management of pulmonary zygomycosis. As much devitalized tissue and necrotic debris as possible is removed. Early recognition of rhinocerebral zygomycosis may reduce or eliminate the need for disfiguring surgery.

Reconstructive surgery may be required for those patients who survive.<sup>225,227-232</sup> Management of pulmonary zygomycosis often includes thoracotomy and resection of a lesion for diagnostic purposes. At the time of biopsy, a complete lobar or segmental resection of a pulmonary zygomycotic lesion restricted to one region of the lung may be as effective in controlling progression of pneumonia as high-dose amphotericin B.<sup>233</sup>

The treatment of choice for zygomycosis is amphotericin B (see Table 27-4). Lipid formulations have supplanted traditional formulations of amphotericin B as first-line therapy due to their decreased nephrotoxicity. Their improved safety profile allows for longer duration of therapy and therefore enhanced efficacy. A reasonable initial starting dosage for lipid formulations of amphotericin B is 5 mg/kg/day.<sup>234-239</sup> Nevertheless, the optimal duration and dosage of amphotericin B for treatment



of zygomycosis are unknown. Therapy should be individualized according to the patient's clinical response and the rate of clearance of the infection, but many patients require a minimum of 6–8 weeks of therapy.

Voriconazole does not exhibit significant activity against the zygomycetes. There have been numerous case reports of the development of breakthrough zygomycotic infections in patients on voriconazole therapy.<sup>234,240–246</sup> In a case–control prospective surveillance study reported by Kontoyiannis et al, 15 of 27 patients with zygomycosis received voriconazole prior to their diagnosis. Voriconazole prophylaxis was found to be an independent risk factor for a zygomycotic infection.<sup>242</sup> The underlying basis for this association is unknown, but may simply be due to the fact that the spectrum of voriconazole does not include the zygomycetes.<sup>234</sup> Posaconazole, an orally administered triazole, may be effective in the salvage treatment of zygomycosis.<sup>247</sup> A 2006 retrospective study of posaconazole as salvage or alternative therapy in 91 patients with zygomycosis conducted by van Burik et al reported complete or partial response in 60% of patients.<sup>248</sup> However, its specific role as primary, adjunctive or salvage therapy in management of zygomycosis needs to be further defined.

In vitro studies have demonstrated that the echinocandins have very little activity against zygomycetes.<sup>249–251</sup> However, echinocandins may play an adjunctive role in the treatment of zygomycosis in the future. A recent study involving a murine model of disseminated zygomycosis caused by *R. oryzae* published by Ibrahim et al demonstrated that caspofungin inhibited (1,3)- $\beta$ -D-glucan synthase.<sup>252</sup> Another study of *R. oryzae* infection in mice with diabetic ketoacidosis also demonstrated improved survival in the group receiving caspofungin in addition to amphotericin B lipid complex compared to those receiving monotherapy with either drug.<sup>253</sup> A recent retrospective study found that the combination of echinocandin plus lipid formulation of amphotericin B improved outcome in patients with zygomycosis.<sup>254</sup>

The role of deferoxamine in increasing iron availability to the zygomycetes and potentiating their pathogenicity has prompted intensive study to determine if other iron chelators can be helpful additions to traditional antifungal therapy. Encouraging studies involving animal models of disseminated *R. oryzae* and zygomycosis demonstrated markedly increased survival in the groups treated with deferiprone, an iron-chelating agent approved in India and Europe for the treatment of iron overload.<sup>251,255,256</sup> In addition, a striking reversal of disease progression was recently reported in a patient with rhinocerebral zygomycosis after the addition of deferasirox as empiric salvage therapy to a previous regimen of high-dose liposomal amphotericin B.<sup>257</sup> Deferasirox (Exjade, Novartis) is currently approved in the US for treatment of iron overload in patients who are transfusion-dependent.

The correction of ketoacidosis in patients with diabetes and reversal of immunologic deficits in immunocompromised patients is a vital component of therapy (see Table 27-5). Recovery from granulocytopenia is essential for survival of patients with rhinocerebral and pulmonary zygomycosis. Recovery from granulocytopenia may occur spontaneously or may be promoted by hematopoietic growth factors, such as G-CSF and GM-CSF. When zygomycosis is documented, corticosteroids should be reduced in dosage or discontinued, where possible. In patients with zygomycosis complicating solid organ

transplantation, HSCT, and neoplastic diseases, the need to treat the underlying disease and the immunosuppressive effects of that treatment are opposing forces that often create a therapeutic dilemma. Zygomycosis ultimately can seldom be cured in a patient with hematologic neoplastic disease without successful induction of remission.

High atmospheric pressures of oxygen or lengthy exposures to hyperbaric oxygen (HBO) are fungicidal in vitro.<sup>258</sup> More clinically relevant shorter and lower pressures (1–3 atmospheres) are fungistatic in vitro, suggesting that HBO may be a potential adjunct in treatment of zygomycosis. Among six patients receiving adjuvant HBO with amphotericin B and surgery, four recovered completely within 1–3 months; the other two patients died. As this study was not randomized, the authors recommend further investigation, possibly in the setting of a randomized trial, of this potential adjuvant modality for treatment of rhinocerebral zygomycosis. Other reports also suggest that HBO may have a beneficial effect in management of this infection.<sup>259,260</sup> A review by Yohai et al of 145 patients with rhinocerebral zygomycosis underscores favorable prognostic factors that are very similar to those with pulmonary involvement, namely early diagnosis, aggressive surgical debridement, optimization of the immunosuppressive regimen, and hyperbaric oxygen therapy.<sup>261</sup>

## Fusarium infections

*Fusarium* spp. are being increasingly recognized as causative agents of disseminated infection, particularly in granulocytopenic patients undergoing intensive antileukemic chemotherapy or HSCT. *F. solani*, *F. oxysporum*, *F. moniliforme*, and *F. chlamydosporum* have been reported to cause disseminated infection in immunosuppressed patients. The lung, sinuses, and skin are the primary portals of entry.<sup>262</sup> The periungual regions of the toes notably may be a particularly important site of initial invasion. Invasive *Fusarium* infections produce a pattern similar to that of invasive aspergillosis.<sup>263,264</sup> *Fusarium* infections in granulocytopenic patients are characterized by pulmonary infiltrates, cutaneous lesions, positive blood cultures, and sinusitis.<sup>265</sup> Biopsy of the cutaneous lesions often reveals fine, dichotomously branching, acutely angular, septate hyphae. Unlike *Aspergillus* spp., *Fusarium* spp. are frequently detected by advanced blood culture detection systems, such as lysis centrifugation.

Traditionally, this emerging fungal pathogen often responded only to high doses of conventional amphotericin B (1–1.5 mg/kg) plus flucytosine.<sup>266</sup> More recently, there have been important new developments in understanding the epidemiology, host defenses, and treatment of *Fusarium* infections. Anaissie described the MD Anderson experience of 43 cases of invasive fusariosis in patients with hematologic malignancy between 1986 and 1995. He emphasized the role of the skin as a potential portal of entry, the response of patients to adjuvant neutrophil transfusions from G-CSF stimulated donors, the potential recurrence of infection during periods of neutropenia, and the importance of the hospital water system as a reservoir of infection.<sup>113,114,267</sup>

Lipid formulations of amphotericin B and voriconazole are now commonly used as first-line therapy for *Fusarium*

infections (see Table 27-4). The data supporting the use of lipid formulations of amphotericin B and voriconazole are based on salvage studies in patients refractory to or intolerant of standard therapy.<sup>238</sup> In a recent study, voriconazole had an efficacy rate of 45% in the salvage treatment of fusariosis.<sup>268</sup> Posaconazole has also been found to be effective as salvage therapy for invasive fusariosis, with a reported success rate of 48%.<sup>269</sup>

Given the suboptimal response of many patients to monotherapy, there has been much interest in combination therapy for the treatment of disseminated fusariosis. Synergy between caspofungin and amphotericin B has been demonstrated in vitro<sup>270</sup> and a few cases of response to this combination of agents have recently been reported.<sup>271,272</sup>

## Respiratory infections due to *scedosporium* species

*Scedosporium apiospermum*, or its teleomorph (sexual stage) *P. boydii*, causes sinusitis, pneumonia, and disseminated infections in immunocompromised hosts and mycetoma in immunocompetent patients. Deeply invasive infections due to *S. apiospermum* have been reported to carry a high mortality.

Pneumonia due to *S. apiospermum* is clinically indistinguishable from that due to *Aspergillus* spp. As with pulmonary aspergillosis, dissemination complicating *S. apiospermum* pneumonia often involves the central nervous system. Diagnostic procedures and approaches, including thoracic CT scan, BAL, and lung biopsy, are also similar to those for invasive pulmonary aspergillosis. The organism in tissue and direct smears resembles *Aspergillus* spp., as angular, septate, dichotomously branching hyphae. However, terminal annelloconidia may be observed histologically in some infected tissues. The definitive microbiologic diagnosis is established by culture, in which the organism may grow as the synanamorph (asexual form) *S. apiospermum* or as the teleomorph *P. boydii* with cleistothecia.

Infections due to *S. apiospermum* are frequently refractory to antifungal chemotherapy, including amphotericin B. Whether this refractoriness reflects impaired host response or intrinsic microbiologic resistance to antifungal compounds is not clear. Antifungal azoles are active in the treatment of infections caused by *S. apiospermum* (see Table 27-4). In vitro assays have demonstrated voriconazole's activity against *S. apiospermum* isolates compared with amphotericin, micafungin, itraconazole, posaconazole and terbinafine.<sup>273-275</sup> There are also published reports of successful treatment of invasive *S. apiospermum* with voriconazole in immunocompromised hosts.<sup>268,276</sup> There are limited data evaluating the newer azoles and echinocandins in the treatment of pseudallescheriasis, but preliminary findings indicate a potential role for posaconazole and ravuconazole.<sup>250,274,277-280</sup>

Unfortunately, immunocompromised patients with pneumonia, cerebral abscesses, endophthalmitis, osteomyelitis or disseminated infections due to *S. apiospermum* often fail to respond to single-agent azole therapy. The efficacy rate for scedosporiosis treatment with voriconazole was 30% in a recent multicenter prospective study.<sup>268</sup> To date, the utility

of combining antifungal agents to treat these infections is unknown.<sup>251</sup>

*Scedosporium prolificans* can also cause pneumonia and invasive disseminated infection in immunocompromised hosts. *S. prolificans* has been considered more clinically virulent than *S. apiospermum*.<sup>281</sup> In a comprehensive literature review of 435 cases, Cortez et al observed that there was a higher mortality rate among patients with infections caused by *S. prolificans* compared to patients with *S. apiospermum* or *P. boydii* infections, by univariate analysis ( $P < 0.001$ ).<sup>282</sup> *S. prolificans* tends to be refractory to currently available antifungal regimens in vitro and in vivo. Surgical debridement and resection as well as reversal of immunosuppression continue to be crucial elements of treatment in most cases.<sup>283</sup>

## Respiratory infections due to dimorphic fungi

*Histoplasma capsulatum* var. *capsulatum*, *B. dermatitidis*, *Coccidioides posadasii* and *C. immitis*. *P. brasiliensis* and *P. marneffei* are endemic dimorphic fungi that may infect the respiratory tract as a primary portal of entry. *Sporothrix schenckii*, while manifesting the typical thermal dimorphism of the endemic dimorphic organisms, does not appear to follow a geographically defined endemic pattern of distribution. Instead, infections due to *S. schenckii* are distributed worldwide but occupy natural niches within woody plants and sphagnum moss. Given its dimorphic mycologic properties, sporotrichosis will be discussed in this section.

Fungal dimorphism is defined here as the temperature-independent phenotypic duality of forms of a fungus. In the inanimate environment at temperatures below 35°C, they produce a mycelial form with hyaline, branching, septate hyphae. The hyphae of *H. capsulatum*, *B. dermatitidis*, *P. brasiliensis*, and *P. marneffei* will convert to budding yeast cells in tissue or on enriched media at 37°C in the laboratory. *Coccidioides posadasii* and *C. immitis*, that is the *C. immitis* complex, produces spherules in tissue.

Endemically dimorphic fungi also share several other features. Each fungus normally exists in nature and has a characteristic geographic distribution, which defines the areas that are endemic for infection. The vast majority of infections with any of these fungi are initiated by inhalation of conidia in nature. The pulmonary infection may be asymptomatic and resolve spontaneously, but reactivation may occur subsequently. Any one of these fungi may disseminate from the lungs to other organs. The rate of infection is high in the specific geographic areas of endemicity but the preponderance of these endemic infections is self-limiting. These fungi routinely infect persons with apparently normal immunity and hence are termed primary fungal pathogens.

In most cases, the natural history of endemic dimorphic mycoses is initiated when aerosolized conidia are inhaled. Upon entry into the lower respiratory tract, the interaction of host defenses and various fungal factors determines the outcome of infection.<sup>284</sup> The fungi are usually contained by alveolar macrophages, which are modulated by T lymphocytes, resulting in a localized granulomatous inflammatory response for *H. capsulatum*, *P. brasiliensis*, and *P. marneffei*.<sup>285-288</sup> A combined acute (pyogenic) and chronic (mononuclear/macrophage)

inflammatory response is often observed with *C. immitis* complex and *B. dermatitidis*.<sup>289,290</sup> Calcifications develop at the site of the resolving granulomatous foci of *H. capsulatum* and are often seen on chest radiographs of infected patients.

More than 95% of cases of histoplasmosis, coccidioidomycosis and paracoccidioidomycosis are estimated to be self-limiting and produce a minimum of symptoms. In most cases, the only evidence of infection is the development of an immune response, which is manifested by the acquisition of a positive delayed-type skin test and the production of specific antibodies, development of precipitins and complement-fixing antibodies, as well as conversion to positive skin tests.<sup>291-293</sup> The small percentage of these episodes that advance to progressive pulmonary infection or clinically overt disseminated infection are often associated with predisposing risk factors, particularly underlying defects in cell-mediated immunity, such as those encountered in HIV-infected hosts or patients receiving corticosteroids.

## Clinical manifestations, laboratory diagnosis, and treatment

The dimorphic fungi that cause systemic mycoses are identified by direct microscopic examination of specimens, by isolation and characterization of the fungus in cultures, by DNA probing of isolates or by urinary and serum histoplasma antigen.

### Histoplasmosis

The clinical manifestations of histoplasmosis may be classified according to site (pulmonary, extrapulmonary or disseminated infection), by duration of infection (acute, subacute, and chronic), and by pattern of infection (primary versus reactivation). Excellent clinical reviews have recently been published.<sup>294,295</sup>

Acute primary pulmonary histoplasmosis (APPH) may develop in a normal, immunocompetent host who is exposed to a heavy inoculum.<sup>296,297</sup> A history of potential environmental exposure, particularly in patients from endemic areas, is sought in patients with APPH. Local public health authorities should be notified if a putative source is identified. The symptoms of APPH, which often resemble those of an influenza-type illness, are usually self-limiting, often being managed with general supportive care. The chest radiograph in APPH typically demonstrates a diffuse alveolar-interstitial infiltrative or reticulonodular pattern. These radiographic changes may resolve completely or leave a fine miliary pattern of pulmonary calcifications. In patients with active pulmonary histoplasmosis, yeast cells of *H. capsulatum* may be observed on direct examination of sputum, often within pulmonary alveolar macrophages. Chronic cavitary pulmonary histoplasmosis (CCPH) is an indolent but progressive respiratory infection of patients with underlying chronic obstructive pulmonary disease. As a group, these patients are usually elderly smoking males who have worked in endemic areas, often in coal-mining regions, who suffer from progressive deterioration of pulmonary function. The progressive loss of pulmonary function is likely a combination of both chronic lung disease and histoplasmosis.

Most cases of asymptomatic infection due to *H. capsulatum* var. *capsulatum* have a clinically asymptomatic fungemia, as evidenced by splenic calcifications, as well as asymptomatic

pulmonary calcifications on chest radiographs. This “cryptic dissemination” to multiple organs permits subsequent reactivation at pulmonary and extrapulmonary sites if the host becomes immunocompromised or similarly stressed, resembling the pathogenesis of tuberculosis. Histoplasmosis may reactivate years later in extrapulmonary tissues, particularly the CNS, adrenal glands, mucocutaneous surfaces, and other sites.<sup>297,298</sup> This pattern of histoplasmosis, which often occurs in elderly and immunocompromised patients, must be differentiated from other mycoses, tuberculosis or neoplastic disease.<sup>299,300</sup> Tissue from any of these sites may be submitted for culture and histopathologic studies.

Disseminated histoplasmosis may develop in immunocompromised patients with cellular immunodeficiencies.<sup>301-304</sup> In these patients, signs of disseminated infection (hypotension, hepatosplenomegaly, pancytopenia, and hypoadrenalism) may overshadow the pulmonary involvement.<sup>305</sup> Disseminated histoplasmosis may also develop in otherwise apparently healthy infants less than 2 years of age. Specimens for culture include blood, urine, bone marrow, and sputum. HIV-infected patients with disseminated histoplasmosis may have multiple necrotizing cutaneous or oral lesions. Biopsy and culture of these lesions may reveal poorly formed granulomas containing an abundant amount of small budding yeast forms due to *H. capsulatum*.

Direct examination of specimens for *H. capsulatum* is best accomplished with special stains. The budding yeast cells of *H. capsulatum* (2–4 µm) on a Calcofluor white or KOH preparation of sputum may be too small for reliable detection and may be confused with *Candida glabrata*, which is similar in size and shape and often colonizes the human oropharynx. The small yeast cells of *H. capsulatum* are observed frequently within the cytoplasm of macrophages. In contrast, the yeast cells of *C. glabrata* are seldom found within macrophages. Giemsa and hematoxylin and eosin (H&E) stains reveal the intracellular yeasts of *H. capsulatum* more readily, especially in sputum, blood smears, bone aspirates, and biopsy specimens. The GMS stain delineates the yeast cells but not the cellular detail of the host inflammatory cells.

Histopathologic examination of paraffin-embedded specimens by H&E and periodic acid-Schiff (PAS) stains reveals that *H. capsulatum* elicits a granulomatous inflammatory response. Large numbers of the tiny yeasts pack the cytoplasm of macrophages in acute pulmonary or disseminated histoplasmosis. The yeast cells of *H. capsulatum* must be distinguished from cells of the intracellular parasites *Leishmania donovani* and *Toxoplasma gondii*. *Leishmania donovani* contains a kinetoplast, which is not present in the yeast cells of *H. capsulatum*. The tachyzoites of *T. gondii* are not stained by GMS. As lesions become fibrotic and calcified, the number of yeasts of *H. capsulatum* continues to diminish. The GMS stain is preferable for detection of the small numbers of yeasts. Budding may not be observed in the chronic lesions of histoplasmosis.

Detection in serum and urine of a carbohydrate antigen of *H. capsulatum* is a valuable tool in diagnosis and therapeutic monitoring of disseminated histoplasmosis, particularly in HIV-infected patients.<sup>303,306</sup> By comparison, antigen detection in serum and urine of non-HIV infected patients with localized pulmonary disease is less sensitive.<sup>307</sup>

PCR assays currently under development also hold promise for the rapid diagnosis of histoplasmosis. Complement



fixation antibody tests and immunodiffusion assays also may be utilized in the diagnosis of infections due to *H. capsulatum*. A fourfold rise in antibody titer to *H. capsulatum* can be diagnostic in patients with chronic and acute pulmonary histoplasmosis.<sup>308</sup>

Treatment of pulmonary histoplasmosis depends upon the host and patterns of disease (see Table 27-4). Immunocompetent patients with acute pulmonary histoplasmosis usually have self-limiting disease, which may be managed with supportive care. Patients with acute pulmonary histoplasmosis who may be elderly, very young (<2 years old), debilitated or immunocompromised may be treated with itraconazole. Profoundly immunocompromised patients or those with severe pulmonary histoplasmosis with hypoxemia, hypercarbia or life-threatening extrapulmonary disease are treated with amphotericin B. The lipid formulation is preferred. After substantial clinical improvement has been achieved, patients can then complete their course of therapy with itraconazole. Duration of treatment is based on radiographic and clinical resolution of the disease, but averages about 3 months in most patients.<sup>295,308</sup>

A recent randomized controlled trial comparing conventional and liposomal amphotericin B in the treatment of disseminated histoplasmosis in AIDS patients demonstrated that use of the latter resulted in improved survival and more rapid resolution of symptoms with less toxicity.<sup>309</sup> Again, therapy may be continued with oral itraconazole when the patient's condition improves. Therapeutic response, particularly in HIV-infected patients who have a high *H. capsulatum* carbohydrate antigen burden, can be monitored by serial urine and serum samples, as measured by radioimmunoassay. Recent data suggest that an enzyme-linked immunoassay is equally sensitive and specific and may be an acceptable alternative for measuring *Histoplasma* antigen levels in urine without the use of radioactive isotopes.<sup>310</sup>

Length of therapy is variable, depending on the rapidity of disease resolution and the patient's immune status. The minimum treatment duration is 6 months in a relatively healthy host, but may be substantially longer.<sup>308</sup> In the past, patients with AIDS were placed on lifelong maintenance itraconazole therapy to prevent relapse of the histoplasmosis infection. Due to the success of highly active antiretroviral therapy (HAART), this is no longer the case; patients with AIDS do not require routine maintenance azole prophylaxis if their CD4 count remains greater than 200 for at least 1 year.<sup>308,309</sup>

### Coccidioidomycosis

The incidence of pulmonary coccidioidomycosis may increase strikingly in endemic areas in the United States due to altered climatic conditions.<sup>311,312</sup> There are two distinct *Coccidioides* species, *C. immitis* and *C. posadasii*.<sup>313</sup> *C. immitis* is primarily associated with infections acquired in the San Joaquin Valley of California, while *C. posadasii* can be isolated in affected patients outside this area. Despite being genetically separate entities, no significant difference in their clinical expression or response to therapy has been reported to date.<sup>314</sup> Clinical manifestations of coccidioidomycosis have been classified into three general groups: (i) initial pulmonary infection, which is usually self-limiting, (ii) pulmonary complications; and (iii) extrapulmonary disease.<sup>315-317</sup>

Primary infections in normal hosts usually resolve spontaneously without antifungal therapy. The presence of erythema

nodosum in an immunocompetent patient with pulmonary coccidioidomycosis signifies a favorable host response and good prognosis. However, primary pulmonary infection, particularly in immunocompromised patients, may evolve into one of several complications: pulmonary nodules, thin-walled cavities, progressive pneumonia, pyopneumothorax, and bronchopleural fistula. Certain patient populations with defective cellular immunity, such as those with HIV infection and those receiving corticosteroids, are more susceptible to progressive pneumonia, complicated pneumonia, and dissemination. Infliximab, a TNF- $\alpha$  antagonist often utilized in the treatment of inflammatory arthritis and autoimmune diseases, has recently been shown to increase the risk of symptomatic coccidioidomycosis.<sup>318</sup>

Dissemination to extrapulmonary sites may result in cutaneous and soft tissue infection, osteomyelitis, arthritis, and meningitis. BAL fluid, percutaneous needle biopsy, and transbronchial biopsy specimens may be submitted to the clinical microbiology laboratory for microscopic examination, cytology, and culture.<sup>319,320</sup> Cerebrospinal fluid and biopsies of other tissues infected by *C. immitis* can be handled in the same manner. More detailed discussion of the clinical manifestations of coccidioidomycosis may be found in several informative reviews.<sup>315,317,321-323</sup>

Due to the risks to laboratory personnel when working with the filamentous form of *C. immitis*, direct examination of sputum, exudates, and tissue is highly recommended. Mature spherules are thick-walled, usually 20–60  $\mu$ m in diameter, and easily recognized on wet mounts using KOH or Calcofluor white. Endoconidia (2–4  $\mu$ m) can be observed in intact or recently disrupted spherules. Hyphae may develop in chronic cavity and granulomatous lesions of pulmonary coccidioidomycosis or in a pleural space having low CO<sub>2</sub> content.<sup>324</sup>

*C. immitis* grows readily on conventional media at 25–30°C usually within 1 week as a floccose buff to yellow to tan colony composed of hyaline, septate hyphae with arthroconidia. Rapid diagnosis of coccidioidomycosis with a DNA probe to ribosomal RNA has been demonstrated by Beard et al.<sup>325</sup>

Coccidioidomycosis histologically is characterized by a variable inflammatory response ranging from an acute pyogenic to a chronic granulomatous reaction. This variability may be due to an acute inflammatory reaction to endoconidia after rupture of spherules. A granulomatous response is observed in association with intact spherules. Spherules are sparse in tissue from patients with resolving infection, but are numerous during progressive disease. Spherules of *C. immitis* are identified easily in tissue by routine H&E, GMS, and PAS stains, particularly the latter.

Immunocompetent patients with self-limiting pulmonary coccidioidomycosis have been managed with observation only (see Table 27-4). However, patients with any form of immunosuppression or debilitation prudently warrant antifungal therapy. The advent of the azoles ketoconazole, itraconazole and fluconazole has permitted a wider range of patients to be treated for coccidioidomycosis for prolonged periods without the toxicity of amphotericin B<sup>326-328</sup> but relapse of infection after any of these treatments is common<sup>329</sup> and in one series was associated with negative serial coccidioidin skin tests and a peak complement fixation titer  $\geq 1:256$ .<sup>330</sup> Fluconazole was shown to be only 55% effective in 40 patients with pulmonary

coccidioidomycosis in an NIAID/Mycoses Study Group trial.<sup>331</sup> Recent data suggest that itraconazole may be slightly superior to fluconazole for treatment of non-meningeal coccidioidomycosis.<sup>332</sup> Conventional amphotericin B is currently recommended for treatment of progressive pulmonary coccidioidomycosis in hospitalized patients who are acutely ill, and those who fail azole antifungal therapy. Experience in the treatment of pulmonary coccidioidomycosis with lipid formulations of amphotericin B is scant, but a recent comparative study utilizing a murine model of coccidioidomycosis suggests that the lipid preparations of amphotericin B have similar efficacy.<sup>333</sup>

The role of the newer triazole antifungal agents such as voriconazole, posaconazole, and ravuconazole is still undefined, although there is a published anecdotal report indicating that voriconazole was useful in refractory cases of coccidioid meningitis.<sup>334</sup> A recently published multicenter trial of 20 patients with chronic pulmonary or non-meningeal coccidioidomycosis demonstrated that posaconazole was effective in 85% of patients.<sup>335</sup>

### Blastomycosis

The manifestations of infection with *B. dermatitidis* are protean: asymptomatic disease, a brief influenza-like illness, self-limited, localized pneumonia in immunocompetent patients, subacute to chronic respiratory illness and fulminant infection with adult respiratory distress syndrome (ARDS).<sup>336,337</sup> The infiltrates of pulmonary blastomycosis are non-specific and appear as a bronchopneumonia or segmental consolidation. These lesions in non-immunocompromised patients may persist for several months and lead to evaluation for chronic pneumonia or pulmonary neoplasm.<sup>338</sup> A subset of patients may present with a more fulminant course of pulmonary blastomycosis with diffuse multilobar involvement and acute deterioration of respiratory function.<sup>339</sup> Chronically progressive blastomycosis may be complicated by dissemination to one or more organs, including the skin, genitourinary tract, bone or central nervous system in immunocompromised patients.<sup>336-342</sup>

Concomitant cutaneous lesions may be ulcerative or verrucous and resemble a variety of chronic infections or skin cancer. Biopsy demonstrates pseudoepitheliomatous hyperplasia, acanthosis, and intraepidermal and dermal abscesses containing blastoconidia of *B. dermatitidis*. Osteomyelitis develops in up to one-third of patients with blastomycosis. The genitourinary tract, especially the prostate and epididymis, is another target of blastomycosis. Urine collected for culture after prostate massage may also reveal *B. dermatitidis*. Meningitis due to blastomycosis is uncommon, often presenting as a basilar process, and difficult to diagnose by culture of lumbar cerebrospinal fluid; recovery of *B. dermatitidis* may be improved with culture of ventricular or cisternal fluid.<sup>343</sup>

Sputum samples, BAL fluid or lung biopsies may be submitted for microscopy and culture. Sputum cytology collected to identify malignant cells in patients with chronic pulmonary infiltrates suspected to be neoplastic may reveal unsuspected yeast cells of *B. dermatitidis*. Lung biopsy may reveal a pyogranulomatous reaction with marked fibrosis. The pseudoepitheliomatous hyperplasia and desmoplastic reaction of pulmonary blastomycosis may simulate bronchogenic squamous cell carcinoma. Unless special stains for fungi are utilized on such tissue, conventional H&E stains may not detect

the presence of organisms. The presence of concomitant bony lesions may lead to an erroneous diagnosis of squamous cell carcinoma of the lung with bony metastases unless cultures and special stains are performed.

Direct Calcofluor white or KOH mounts of sputum, exudates, and tissues can demonstrate the yeast cells of *B. dermatitidis*, which are large, spherical, and thick walled and measure approximately 8–15 µm in diameter. The yeast cells bud singly and have a wide base of attachment between the bud and parent yeast cell. The bud of *B. dermatitidis* often attains the same size as the parent yeast before becoming detached. Infected tissues stained with GMS will reveal these characteristic yeast forms. These yeast cells have also been recognized on cytologic specimens of sputum (often submitted to rule out primary lung cancer) treated with Papanicolaou stain.

Treatment of pulmonary blastomycosis has been greatly advanced by the use of itraconazole (see Table 27-4).<sup>328,344</sup> A minimum 6-month course of itraconazole is recommended for immunocompetent patients with mild to moderate pulmonary blastomycosis. Voriconazole may be utilized as alternative therapy for this subset of patients.<sup>345</sup> A formulation of amphotericin B should be administered to patients who cannot tolerate azoles or for whom azole therapy is contraindicated. In addition, it should be used in patients who are unresponsive to azole therapy and in immunocompromised patients with pulmonary blastomycosis. After the initial course of amphotericin B succeeds in ameliorating the disease process, therapy may be switched to itraconazole.<sup>346</sup> With prompt diagnosis by microscopic examination of tracheal secretions, intensive therapy with amphotericin B, and ventilatory support, good recovery from overwhelming pulmonary blastomycosis associated with the adult respiratory distress syndrome is possible. Itraconazole should be used indefinitely for secondary prophylaxis against recurrent blastomycosis in patients with AIDS.<sup>347</sup> Posaconazole<sup>348</sup> and nikkomycin Z<sup>349</sup> have demonstrated in vitro and in vivo activity against *B. dermatitidis*.

### Paracoccidioidomycosis

Infections due to *P. brasiliensis* arise endemically in Central and South America. However, within this vast area, the endemicity varies considerably.<sup>350,351</sup> More than 95% of patients who progress to symptomatic paracoccidioidomycosis are males, possibly because of estrogen-mediated inhibition of mycelial-to-yeast transformation.<sup>352,353</sup> Estrogen-binding proteins have been detected in the cytoplasm of the organism and as a result of estradiol incorporation, new proteins are produced during the transformation.<sup>350</sup> Paracoccidioidomycosis is characterized by a depressed cellular and activated humoral immune response. In experimental models of infection, interferon-γ activity<sup>351-353</sup> and monocyte adherence to *P. brasiliensis*<sup>354</sup> are important host defense mechanisms.

Paracoccidioidomycosis is classified into three patterns of infection: acute pneumonia, chronic pneumonia, and disseminated infections.<sup>355,356</sup> These infections may be further classified as primary infection or reactivation. Fever, cough, sputum production, chest pain, dyspnea, hemoptysis, malaise, and weight loss may occur with pneumonia and disseminated infection. Extrapulmonary lesions often develop on the face and oral mucosa. Other sites include lymph nodes, spleen, liver, gastrointestinal tract, and adrenal glands. The epidemiology

and clinical manifestations of paracoccidioidomycosis are discussed in greater detail elsewhere.

*Paracoccidioides brasiliensis* is identified by direct examination of sputum or BAL fluid, scrapings from mucocutaneous ulcers or tissue biopsies to reveal variations of the characteristic thick-walled pilot wheel or mariner's wheel configuration of the yeast form. The parent yeast cell measures 15–30 µm in diameter, whereas the buds are 2–10 µm and have a narrow base of attachment. The presence of multiple budding distinguishes this organism from *C. neoformans* and *B. dermatitidis*. Histopathologic examination using H&E, GMS, and PAS stains of tissue infected with *P. brasiliensis* reveals a pyogranulomatous process with infiltrating polymorphonuclear leukocytes, mononuclear cells, macrophages, and multinucleate giant cells.

Treatment of paracoccidioidomycosis with trimethoprim-sulfamethoxazole, ketoconazole or itraconazole has been highly successful. Amphotericin B may be used for refractory or severe infections (see Table 27-4).

### Penicilliosis

Increasingly recognized as a cause of disseminated infection in HIV-infected patients from Southeast Asia, *P. marneffei* has emerged as an important endemic pathogen.<sup>357,358</sup> Little is reported about the pulmonary manifestations of disseminated penicilliosis but the infection has a striking resemblance to disseminated histoplasmosis in HIV-infected patients. Among 92 recently reported patients from Chiang Mai province in Northern Thailand, the most common presenting symptoms and signs were fever (92%), anemia (77%), weight loss (76%), and skin lesions (71%); 87% of patients presenting with skin lesions had generalized papules with central umbilication. The diagnosis of *P. marneffei* is readily established by blood culture or via direct smear and culture of umbilicated centrally necrotic lesions, bone marrow aspirate or peripheral lymph nodes. Such diagnostic measures are preferred before pursuing a more invasive procedure, such as BAL.

Itraconazole and amphotericin B have been proven to be effective single agents in the treatment of disseminated *P. marneffei* infection (see Table 27-4). The recommended treatment regimen for HIV patients with disseminated penicilliosis consists of a 14-day course of amphotericin B (0.6 mg/kg/day) followed by 10 weeks of itraconazole (400 mg/day).<sup>359,360</sup> This regimen was well tolerated and successful in treatment of 72 of 74 patients. In an earlier study, itraconazole as monotherapy for disseminated *P. marneffei* infection required almost 2 months for successful eradication of positive fungal blood cultures.<sup>359,361</sup> A randomized placebo-controlled trial of itraconazole significantly reduced the frequency of relapsed penicilliosis in HIV-infected patients following initial induction therapy.<sup>358</sup> Itraconazole secondary prophylaxis may be safely discontinued in patients receiving HAART when CD4 cell counts exceed 100 cells/µl for 6 months.<sup>362</sup> In addition, primary prophylaxis with oral itraconazole may be indicated in patients with CD4 counts less than 200 cells/µl who reside in areas of Southeast Asia where the disease is frequently seen.<sup>359,363</sup>

### Sporotrichosis

Pulmonary sporotrichosis is an unusual infection, which is principally observed in older males with chronic obstructive pulmonary disease; they may also have a history of alcohol

abuse or diabetes mellitus, which may further add subtle immune impairment. Patients with AIDS are also at risk for pulmonary sporotrichosis. It usually presents as a chronic cavitary pneumonia typically in an upper lobe distribution.<sup>364</sup> The differential diagnosis of thin-walled upper lobe cavities of pulmonary sporotrichosis should include coccidioidomycosis, tuberculosis, and histoplasmosis.<sup>365</sup>

The initial manifestations of pulmonary sporotrichosis include productive cough, fever, weight loss, anorexia, dyspnea, and hemoptysis. Calcification and hilar lymphadenopathy are unusual. Diagnosis is best established through BAL or by biopsy. Blood cultures are usually negative. Intravenous amphotericin B is the treatment of choice for severe infections, and itraconazole may be used to treat milder cases of pulmonary sporotrichosis (see Table 27-4). Patients with AIDS may require lifelong suppressive therapy with itraconazole following completion of their initial therapy.<sup>366</sup>

## Pulmonary cryptococcosis

Pulmonary cryptococcosis develops in extent and severity according to the level of immune impairment and underlying diseases. Pulmonary cryptococcosis is usually a saprophytic process or limited pulmonary infection in patients with chronic obstructive pulmonary disease, while it is a more aggressive infection leading to disseminated cryptococcal disease in immunocompromised patients, such as organ transplant recipients.<sup>367-369</sup>

Among HIV-infected patients, pulmonary cryptococcosis is frequently associated with disseminated infection. Among HIV-infected patients, fever, cough, dyspnea, and pleural pain are common initial manifestations. Interstitial infiltrates, milary nodules, ARDS, hilar lymphadenopathy, and pleural effusion are typical radiographic features in these patients.<sup>370-373</sup> Extrapulmonary infection, including meningoencephalitis and cutaneous lesions in HIV-infected patients, should be sought in attempting to establish a diagnosis. Cutaneous lesions may resemble molluscum contagiosum. The pulmonary lesions in less compromised patients may simulate metastatic carcinoma.

Cultures of cerebrospinal fluid (CSF), blood, BAL, and skin biopsies are most likely to yield a diagnosis of cryptococcosis. Cryptococcal antigen titers measured by latex agglutination or by EIA in CSF and serum of HIV-infected patients are typically elevated to levels often exceeding 1:1000.<sup>374</sup> Detection of organisms in biopsy specimens can be enhanced by use of mucicarmine or Alcian blue stains of the capsular acid mucopolysaccharide (glucuronoxylomannan).

Treatment of pulmonary cryptococcosis in non-HIV infected patients depends upon the patient's immune status, presence of symptoms, and degree of extrapulmonary involvement. These treatment recommendations are well summarized elsewhere.<sup>375,376</sup> If the patient is HIV infected, an initial course of amphotericin B with or without flucytosine, followed by maintenance therapy with fluconazole, is recommended (see Table 27-4).<sup>377</sup> There is a high propensity for relapse of cryptococcal meningitis in HIV-infected and chronically immunosuppressed patients if maintenance therapy is not continued. However, interruption of maintenance therapy may be considered in HIV patients on HAART who have demonstrated



sustained increases in their CD4 lymphocyte count for more than 6 months. If maintenance therapy is electively discontinued, close follow-up is necessary to ensure that antifungal therapy is quickly started if the cryptococcal infection relapses or if the patient's immune status worsens.<sup>375</sup> Itraconazole may be used as an alternative therapy for patients with mild cases of pulmonary cryptococcosis.<sup>346</sup> The lipid formulations of amphotericin B, in particular ABLC<sup>238,378</sup> and L-AmB,<sup>379</sup> have been efficacious for cryptococcal meningitis but their use in isolated cryptococcal pneumonia has been less well studied. The intralipid formulation of amphotericin B did not confer additional benefit against cryptococcal meningitis in a recent randomized trial and therefore cannot be recommended at this time.<sup>380</sup>

## Pulmonary candidiasis

Pulmonary candidiasis may be a secondary process arising from hematogenous dissemination or rarely a primary bronchopneumonia.<sup>35,381,382</sup> Primary *Candida* bronchopneumonia may be found in severely debilitated patients with solid tumors, neutropenic patients with extensive chemotherapy-induced oral mucositis, and very low birthweight infants. Aspiration of infected oral secretions into the tracheobronchial tree with extension into pulmonary parenchyma is the primary route of infection for *Candida* bronchopneumonia. Hematogenous pulmonary candidiasis is a frequent route of lung infection in neutropenic patients with disseminated infection.

Biopsy of lung tissue is the only reliable means of establishing a diagnosis in patients with pulmonary candidiasis. The presence of *Candida* spp. in the BAL of a patient with pulmonary infiltrates is sufficiently non-specific as to preclude a definitive diagnosis. Treatment of *Candida* bronchopneumonia or hematogenous disseminated candidiasis is initiated with fluconazole, an echinocandin or amphotericin B (see Table 27-4).

## Pulmonary trichosporonosis

Pulmonary and disseminated trichosporonosis are uncommon but frequently fatal infections in neutropenic patients or those receiving corticosteroids.<sup>383</sup> Clinical manifestations are characterized by refractory fungemia, funguria, renal dysfunction, cutaneous lesions, chorioretinitis, and pneumonia. The pulmonary infiltrates of *Trichosporon* pneumonia consist of either bronchopneumonia from aspiration from an oropharyngeal source or multiple nodular pulmonary infiltrates from hematogenous dissemination.

Biopsies of cutaneous lesions generally reveal typical arthroconidia, blastoconidia, pseudohyphae, true hyphae, and vascular invasion. The serum cryptococcal latex agglutination test may be positive due to shared antigens and resultant cross-reactivity between *T. asahii* and *C. neoformans*.

Despite the administration of amphotericin B, fungemia may persist. The organism is inhibited but not killed by safely achievable serum concentrations of amphotericin B. Antifungal triazoles, such as fluconazole, have been found to be active in vivo against this organism. Thus, high-dose fluconazole (10–12 mg/kg/d) and reversal of immunosuppression are the preferred therapy for this infection (see Table 27-4). More

recently, voriconazole has been used successfully in patients with pulmonary and disseminated trichosporonosis. Antifungal therapy under these circumstances requires continuation until resolution of all clinical manifestations of infection. Recurrent infection may nevertheless occur during a subsequent period of cytotoxic chemotherapy.

## *Pneumocystis pneumonia*

*Pneumocystis jiroveci*, formerly known as *P. carinii*, is a major cause of opportunistic infection of the respiratory tract. Pneumonia caused by this organism, known as PCP or PJP, is an AIDS-defining illness that causes significant morbidity and mortality in HIV-infected patients. However, patients with inherited or acquired immune defects and those on immunosuppressive medications are also vulnerable to *Pneumocystis* infection.

The organism was previously considered to be a protozoan but recent data indicate that it may be better classified as a fungus. A 1988 study comparing *P. carinii* ribosomal RNA sequences with that of the fungus *Saccharomyces cerevisiae* indicated that there were many evolutionary similarities between the two organisms.<sup>384</sup> In addition, *Pneumocystis* also has staining characteristics similar to fungi.<sup>385</sup>

A detailed discussion of pulmonary infections caused by *Pneumocystis* is beyond the scope of this chapter and therefore only a limited overview of its clinical characteristics and treatment follows. Although PCP in the setting of AIDS tends to have an indolently progressive course, it has the potential to cause a rapidly progressive, life-threatening pneumonia in a vulnerable host. Patients often present initially with a history of dry cough, fever, dyspnea, and difficulty with deep inspiration. Physical examination of an affected patient typically demonstrates varying degrees of tachypnea and tachycardia, and cyanosis and rales may be evident. Chest radiographs often exhibit the pathognomonic bilateral diffuse infiltrates with a ground-glass appearance; however, focal infiltrates may be seen, especially in patients who are not infected with HIV. The gold standard for diagnosis is a lung biopsy but studies of BAL fluid and induced sputum are more often utilized. *Pneumocystis* can usually be identified by routine staining of these samples. Newer PCR and immunocytochemical techniques may also be beneficial when there is a high index of suspicion despite negative smears.<sup>385,386</sup>

Trimethoprim-sulfamethoxazole (TMP/SMX) has been the treatment of choice for both the prevention and treatment of *Pneumocystis* infection for decades. Adverse reactions to TMP/SMX may include rash, transaminase elevations, fever, and nephrotoxicity. Myelosuppression is another common side effect that often limits its use in already immunocompromised patients. Second-line therapy for patients who cannot tolerate or do not respond to TMP/SMX should consist of intravenous pentamidine, intravenous clindamycin plus primaquine, or dapsone with trimethoprim. Atovaquone may be considered in selected cases, such as those with glucose-6-phosphate dehydrogenase deficiency. Lastly, trimetrexate with leucovorin is reserved for salvage or initial therapy in severe cases of PCP.<sup>385,387,388</sup> Adjunctive therapy with corticosteroids may be considered in patients with significant PCP-induced hypoxemia, as some studies have indicated that

its use is associated with faster recovery.<sup>387,389-391</sup> Dapsone, intravenous or inhalational pentamidine, and atovaquone are alternative prophylactic medications in patients for whom TMP/SMX is contraindicated.

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# Fungal infections of the central nervous system

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## Introduction

Fungal infections involving the central nervous system (CNS) are increasing in parallel with the increasing frequency and diversity of fungal infections as a whole. Despite improvements in diagnostic modalities and the introduction of a number of improved therapeutic agents in recent years, the therapeutic outcome is frequently less than satisfactory. There have been several recent reviews on the subject of fungal infections of the central nervous system.<sup>1-6</sup>

Fungi reach the central nervous system by several different mechanisms. Most commonly, infection results from hematogenous spread, a pulmonary site being the initial focus of infection. Local extension can occur from the paranasal sinuses, the ear or the orbits. Traumatic introduction occurs via:

- surgical procedures
- introduction of foreign bodies in the form of ventriculoperitoneal shunts and Ommaya reservoirs
- performance of lumbar punctures and injection of drugs
- head trauma.

Several different clinical syndromes can result from fungal infections; the specific syndrome may sometimes provide clues to the responsible etiologic agents (Table 28-1).<sup>2</sup> Most of the endemic yeasts, *Coccidioides*, *Histoplasma*, *Sporothrix*, *Blastomyces*, *Cryptococcus*, and those causing nosocomial infections, like *Candida*, cause a *meningitis syndrome*, frequently a subacute or chronic meningitis.<sup>7</sup> Meningitis due to the filamentous fungi is quite rare but does occur. Abscesses are common with certain fungi, including *Aspergillus*, the zygomycetes, *phaeohyphomycosis* etiologic agents and sometimes *Candida* spp. The zygomycetes are notorious for causing rhinocerebral disease, particularly in diabetic patients. Fungi that involve the basilar meninges (*Coccidioides*, *Histoplasma*, *Cryptococcus*, and *Candida*) can cause vasculitis and stroke by involving the local vessels. On occasion, *Candida* and *Histoplasma* can infect the cardiac valves and cause a stroke syndrome from embolic lesions. In addition, there are those organisms that are classically associated with angiotropism (*Aspergillus*, the zygomycetes), that commonly cause infarction and hemorrhagic necrosis. Spinal syndromes are rare but do occur under some situations.

It is critically important that the evaluation of patients who are suspected of having a CNS fungal infection is carried out in an expeditious manner, as the mortality rate is quite high, progression of disease is quite rapid on occasion and the brain is not a particularly forgiving organ. A thorough history may frequently identify host factors that predispose to particular types of fungal infections, including underlying illnesses, immunosuppressive medications (corticosteroids, biologic response modifiers) or hobbies (e.g., spelunking). Many of the responsible fungal agents cause systemic disease; consequently, there may be clues in the mucous membranes, skin, prostate or lungs on physical examination. Specific laboratory tests should be ordered with a hypothesis in mind regarding potential etiologic agents. Because of the peculiarities of antimicrobial susceptibilities for the various fungi, the importance of making a specific microbiologic diagnosis in order to prescribe appropriate therapy cannot be emphasized strongly enough.

## Aspergillosis

Primary central nervous system aspergillosis without disseminated disease or some form of immunosuppressive illness is extremely rare.<sup>8,9</sup> However, CNS aspergillosis occurs in 10–20% of patients with invasive aspergillosis and only rarely is the brain the sole site of infection. Major risk factors for invasive aspergillosis also predispose to CNS disease and include hematologic malignancies, allogeneic bone marrow transplantation, hematopoietic stem cell transplantation, solid organ transplantation, the acquired immunodeficiency syndrome, chronic pulmonary diseases and use of immunomodulating drugs, such as alemtuzumab.<sup>10</sup> Other identified risk factors have included hepatic failure, Cushing's syndrome, and thermal burns.<sup>9,11</sup>

The incidence of CNS disease varies from about 14% in patients with acute leukemia and invasive aspergillosis to about 40–50% in patients undergoing allogeneic bone marrow or hematopoietic stem cell transplantation who have invasive aspergillosis.<sup>12,13</sup> In these two patient groups, immunosuppressive therapy and prolonged chemotherapy-induced neutropenia are the responsible factors predisposing to invasive disease.<sup>8</sup> The onset of invasive aspergillosis after allogeneic bone marrow



**Table 28-1** Epidemiological and clinical correlates of fungal CNS infections

Fungal species	Clinical/epidemiologic setting	Clinical manifestations
<i>Aspergillus</i> spp.	Neutropenia, hematopoietic stem cell transplantation, steroid therapy, late-stage HIV infection	Abscess(es) Chronic meningitis
<i>Blastomyces dermatitidis</i>	Normal host involved in outdoor activities in southeastern, south central and midwestern United States as well as along the St Lawrence River	Chronic meningitis Intracerebral abscess(es)
<i>Candida</i> spp.	Nosocomial settings, complicating ventricular shunts or recent therapy for bacterial meningitis	Multiple microabscesses Macroscopic abscess(es) Subacute meningitis
<i>Coccidioides immitis</i> and <i>posadasii</i>	Dark-skinned races, HIV infection, steroid therapy with residence or travel to Southwest United States, parts of Mexico, Central and South America	Subacute to chronic meningitis Focal intracerebral abscess(es) Cerebral vasculitis
<i>Cryptococcus</i>		
<i>C. neoformans</i>	HIV infection, Steroid therapy, CD4+ lymphocyte depletion syndrome Biologic response-modifying agents	Subacute to chronic meningoencephalitis
<i>C. gattii</i>	Normal host in restricted geographic locations (Australia, Southern California, British Columbia, Washington State)	Focal intracerebral abscess(es) (cryptococcomas)
<i>Histoplasma capsulatum</i>	HIV infection	Chronic meningitis Focal intracerebral abscess(es)
<i>Paracoccidioides brasiliensis</i>	Occupational exposure in parts of Latin America	Focal intracerebral abscess(es)
<i>Scedosporium/Pseudallescheria</i> spp.	Neutropenia Near-drowning	Chronic meningitis Abscess
<i>Sporothrix schenckii</i>	Occupational/environmental exposure ± alcoholism HIV infection	Chronic meningitis
Zygomycetes	Diabetes mellitus with acidosis, neutropenia	Rhinocerebral form
	Parenteral drug addicts	Abscesses in cerebrum and central nuclei

transplantation is bimodal and peaks at approximately 16 and 96 days post transplantation.<sup>14</sup> However, there are suggestions that invasive *Aspergillus* disease may be occurring later than in the past because of changes in transplantation-related practices, such as the use of peripheral blood stem cell transplantation, more frequent application of non-myeloablative regimens for allogeneic stem cell transplantation, and the delayed onset of both graft-versus-host disease and opportunistic viral infections like cytomegalovirus as a result of the use of prophylactic agents.<sup>15</sup>

In a recently published retrospective study that described treatment of CNS aspergillosis in 81 patients, there was definitive microbiology available for 50. Of these, 44 were *Aspergillus fumigatus*, five were *A. nidulans*, two were *A. flavus* and one was *A. terreus*; two patients had dual infections and one had a triple infection.<sup>16</sup>

CNS *Aspergillus* disease may manifest as single or multiple cerebral abscesses, meningitis, epidural abscess or subarachnoid hemorrhage. Parenchymal brain involvement in the form of abscesses, the most common manifestation, occurs

as a result of hematogenous spread, usually from a pulmonary primary source. Occlusion of intracranial vessels ensues with consequent infarction of tissue and subsequent abscess formation.

Presenting manifestation of invasive aspergillosis may be non-specific and complicate diagnosis. Furthermore, patients may present either with a syndrome of meningitis, that tends to be subacute or chronic, or with evidence of parenchymal brain involvement causing focal neurologic deficits and seizures.<sup>9</sup> Patients are frequently febrile but the fever may be due to other etiologies, both infectious and non-infectious. Because of the relative lack of an inflammatory response in patients who are profoundly immunosuppressed, the presentation may be late and include alterations in mental status or seizures, with rapid disease progression, followed shortly afterwards by death.<sup>12</sup> Focal neurologic findings and headaches are more likely to appear in patients who are less severely immunosuppressed and are usually the result of hemorrhagic infarcts.<sup>9,17</sup>

Isolated meningitis is unusual. Cases have been reported in parenteral drug abusers,<sup>18</sup> patients who are neutropenic or diabetic, or those on prolonged steroid therapy. It may present as an extension of paranasal sinus disease, a complication of intrathecal antibiotic therapy or in a postoperative setting after neurosurgery.<sup>19-21</sup> Meningismus or signs of meningeal irritation are unusual but occasionally occur.<sup>9,18</sup> Meningeal infection usually occurs focally adjacent to areas of infection in the cerebral or cerebellar hemispheres.<sup>9</sup>

Central nervous system aspergillosis is relatively uncommon in patients with HIV infection.<sup>22</sup> Mylonakis reported a summary of 38 patients who had CNS aspergillosis complicating HIV infection. All of the patients had very advanced HIV disease and had other factors that predisposed to *Aspergillus* infection, including neutropenia and corticosteroid use. Non-specific neurologic symptoms were the most common presenting manifestations. Three-quarters of the patients had other sites of infection, most commonly lungs, sinuses, ears, and orbits. Medical therapy was unsuccessful in all of the cases.<sup>22</sup>

Computed tomography (CT) typically shows multiple hypodense, well-demarcated lesions; evidence of hemorrhage and mass effects are unusual. However, in individuals with relatively normal white blood cells (WBC) counts, enhancement and surrounding edema are more frequent. Magnetic resonance imaging (MRI) may be more sensitive in detecting subcortical and smaller lesions that do not enhance.<sup>17,23</sup>

Although the definitive diagnosis of cerebral aspergillosis is confirmed by biopsy, the etiology can frequently be inferred in patients with widely disseminated disease. In patients with parenchymal brain involvement, cerebrospinal fluid (CSF) findings are non-specific. Elevations of protein are common, as is the absence of a cellular response. Cultures are routinely negative. In patients with meningitis, there is frequently a polymorphonuclear leukocyte pleocytosis, elevated protein, and hypoglycorrhachia.<sup>18</sup>

In a preliminary study that examined CSF galactomannan levels in five patients with CNS aspergillosis, levels were significantly higher in patients with CNS aspergillosis compared to controls.<sup>24</sup> Calculation of a galactomannan index suggested that from 95.6% to 99.9% of the galactomannan was produced intrathecally. The authors suggested that detection of *Aspergillus* galactomannan in the CSF may be beneficial in the diagnoses of cerebral aspergillosis in patients at high risk for aspergillosis and with a compatible neurologic syndrome.<sup>24</sup>

Despite therapy, mortality in excess of 90% has been regularly reported in patients receiving amphotericin B-based regimens.<sup>12,25-28</sup> Successful outcomes have recently been reported using lipid-based amphotericin B regimens, frequently along with itraconazole, or itraconazole alone. Recent reports have documented substantial penetration of voriconazole into both the CSF and CNS, stimulating the use of that drug for treatment.<sup>29,30</sup> Several reports of successful therapy have appeared with partial or complete response rates ranging from 16% to 42%, which are much higher than those documented with previous regimens.<sup>16,31,32</sup> Recently published guidelines promulgated by the Infectious Diseases Society of America recommend voriconazole as the primary drug of choice for systemic antifungal therapy of CNS aspergillosis; itraconazole, posaconazole or high dosages of a lipid formulation of amphotericin B are recommended for patients intolerant of or refractory to voriconazole.<sup>33</sup>

There are currently insufficient data to advocate performing routine in vitro susceptibility testing for guiding therapeutic decisions in the treatment of CNS aspergillosis. In the retrospective study described above, 50 isolates were subjected to in vitro testing; all isolates studied were susceptible to voriconazole (minimum inhibitory concentration (MIC) range 0.03–0.5 µg/ml; IC<sub>90</sub> 0.5 µg/ml), itraconazole (MIC range 0.03–1.0 µg/ml; IC<sub>90</sub> 0.25 µg/ml), and amphotericin B (MIC range 0.5–2.0 µg/ml; IC<sub>90</sub> 1.0 µg/ml), despite having substantial exposure to several of these agents previously.<sup>16</sup>

The role of combination antifungal therapy in the treatment of invasive aspergillosis has not yet been properly studied, particularly in patients with CNS disease.<sup>33</sup> Non-comparative studies have recently suggested improved efficacy of the combination of voriconazole and caspofungin for recipients of solid organ transplants with invasive aspergillosis, although only one patient with CNS disease was included and the specific response of that patient was not detailed.<sup>34</sup>

In previous reviews, the role of adjunctive neurosurgery has been discouraged, except for diagnostic purposes.<sup>17</sup> However, in selected patients, the successful use of antifungal therapy in combination with neurosurgical interventions has been reported and may be beneficial.<sup>16,34-36</sup> In the review by Schwartz et al,<sup>16</sup> significant survival benefit was demonstrated in patients who received neurosurgical intervention along with antifungal therapy. A number of different neurosurgical procedures were performed, including craniotomy with abscess resection, abscess drainage, ventricular shunting and placement of an Ommaya reservoir. The benefits from surgery may have derived from being able to establish an etiologic diagnosis early, as well as removing non-viable tissue that may interfere with the penetration of antifungal agents. The optimal neurosurgical procedure for CNS aspergillosis is not yet well defined and may depend on the size and location of the abscess and amount of necrotic tissue, as well as timing.

## Candidiasis

Risk factors that predispose to systemic candidiasis also predispose to *Candida* CNS infections,<sup>37</sup> and include:

- prolonged immunosuppression due to hematologic malignancies and transplantation

- intravascular catheters
- burns
- prematurity
- abdominal surgery
- parenteral drug abuse
- chronic granulomatous disease
- neutrophilic myeloperoxidase deficiency
- prolonged antibiotic use
- recent neurosurgery or insertion of CSF derivative device
- AIDS.

Several different pathogenic mechanisms result in CNS candidiasis. Brain microabscesses account for 0.2–0.5% of documented CNS *Candida* infections and result from prolonged candidemia. Up to 50% of patients with systemic candidiasis have CNS invasion.<sup>38–40</sup> The microabscesses are usually found widely disseminated within the brain, particularly at the junctions of the gray and white matter of the basal ganglia and cerebellum. They tend to be less than 0.3 cm in size. Patients suffer from diffuse encephalopathy with progressive confusion and diminished consciousness. Because this presentation is so non-specific, diagnosis is usually only made in 21% of patients antemortem, with neurologic involvement not being documented until autopsy.

Macroabscesses present like cerebral mass lesions with seizures and neurologic focal signs including hemiparesis, aphasia, and visual field defects. They occur most commonly at the parietooccipital level. Patients develop a progressive increase in intracranial pressure.

*Candida* meningitis may present acutely but more typically has a subacute course with evolution over 2–4 weeks. Patients have fever, headache, diminished consciousness, lethargy and confusion. Meningeal signs may be present.<sup>41,42</sup> They may exist concurrently with microabscesses. Invasion of the arteries at the base of the brain can occur and result in vasculitis with thrombosis and secondary infarction, hemorrhage, and hemorrhagic necrosis. Newborns with meningitis may have coincidental bone and joint involvement.

*Candida* meningitis related to neurosurgery was reviewed by Nguyen and Yu.<sup>43</sup> Among 18 cases, direct inoculation into the CNS during surgery by way of an infected wound or ventriculostomy occurred in 13 of 18 (72%) patients. The time between insertion of ventriculostomy devices and infection was 13–36 days. Most patients had recently received antibacterial agents, 50% for bacterial meningitis. The CSF analysis revealed neutrophilic pleocytosis that was indistinguishable from bacterial meningitis. The overall mortality was 11%. In a review of *Candida* meningitis after neurosurgery in 21 patients, 86% had a ventricular shunt.<sup>43</sup> *Candida* spp. were isolated from multiple CSF samples of 10 patients who had been treated, seven of 10 by indwelling devices and nine of 10 by lumbar puncture. In 11 cases *Candida* was the only isolate recovered from the CSF sample; CSF samples obtained by lumbar puncture were negative in 10 of 11 patients. Two symptomatic patients were treated; none of the nine untreated patients died of infection, raising questions regarding the clinical significance of a single positive sample drawn through an indwelling device.<sup>44</sup>

*Candida* should be considered when infection occurs in the presence of a ventriculoperitoneal shunt causing shunt obstruction that results in hydrocephalus and decreased

consciousness.<sup>45,46</sup> On occasion, intestinal perforation by the shunt can result in infection and *Candida* meningitis.

Despite the relative frequency of mucosal disease in patients with HIV infection, systemic disease and meningitis are quite rare. When identified, the meningitis has a subacute evolution over 2–4 weeks or longer, with headache (93%) and fevers (86%). Nuchal rigidity (50%), altered mental status with disorientation and confusion, and cranial nerve abnormalities may also occur. The average CD4 cell count in affected patients is 135/mm<sup>3</sup>.<sup>47</sup> Seventy-one percent had some other factor predisposing to *Candida* meningitis. CSF pleocytosis (<500 cells/mm<sup>3</sup>) was present in virtually all cases; over one-half of cases had neutrophilic predominance. CSF glucose levels were <40 mg/dl in 78% and the median protein level was elevated between 25 and 580 mg/dl. CSF Gram stain demonstrates yeast in up to one-third of cases; culture was positive 79% of the time, although it may require multiple lumbar punctures to yield a positive culture.

Radiographic evidence of microabscesses is usually not possible because their size is below the limits of resolution of CT scans. The cerebrospinal fluid profile is usually normal. When macroabscesses are present, CT and MRI demonstrate ring enhancing lesions, frequently with a hemorrhagic component. CSF examination may have increased protein and moderate pleocytosis but smears and cultures are usually negative.

Cerebrospinal fluid analysis in patients with meningitis may be normal but more typically has an elevated cell count as high as 2000/mm<sup>3</sup> with a predominance of either lymphocytes or neutrophils. Protein values are increased greater than 100 mg/dl. Sixty percent of patients have a CSF glucose value of <40 mg/dl. Forty percent have a positive Gram stain and culture will be positive in 80%.<sup>37,41,42</sup>

The clinical significance of a positive CSF culture for *Candida* has been evaluated by Chen et al.<sup>48</sup> Patients whose CSF grows *Candida* should receive antifungal therapy when they have any of the following:

- an implanted CSF device
- a long-term indwelling central venous catheter
- CSF WBC count >40 cells/mm<sup>3</sup>
- multiple isolations of *Candida* from the CSF
- isolation in an adult after being hospitalized for more than 20 days.

Recommendations for treatment of CNS *Candida* infections are based mostly on observational reports.<sup>49</sup> The drugs of choice for initial therapy include amphotericin B deoxycholate at a dosage of 0.7–1.0 mg/kg daily plus 5-flucytosine at a dosage of 25 mg/kg daily in four divided doses. With this regimen, response rates in the range of 70–88% can be expected.<sup>50,51</sup> Because of the frequency of relapse of this particular disease manifestation, it is recommended that therapy be continued for a minimum of 4 weeks following resolution of all signs and symptoms of infection.<sup>49</sup> There are insufficient data available to make recommendations regarding use of azoles for treating *Candida* CNS infection. Based on experiences in other CNS mycoses, it seems reasonable to include various azoles, like fluconazole or voriconazole, in the treatment armamentarium in appropriate situations.



Prognostic factors for a poor outcome that have been defined in earlier studies include:

- prolonged course before diagnosis
- hypoglycorrhachia
- presence of intracranial hypertension
- the presence of focal neurologic deficits.<sup>41</sup>

Twenty-five percent of patients with CNS candidiasis at autopsy have evidence of vascular invasion of the arterial lumen in cerebral vessels, resulting in vascular infarcts, particularly affecting the basal ganglia.

## Blastomycosis

Central nervous system involvement occurs in approximately 5% of patients with systemic blastomycosis,<sup>52</sup> although in the largest reported series, CNS involvement was found in 8% of cases at autopsy.<sup>53</sup> Disease manifestations may include chronic meningoencephalitis in one-third of patients, intracranial abscess (blastomycoma) in another one-third,<sup>54</sup> and spinal epidural or vertebral abscesses in approximately 20%.<sup>55-59</sup> Primary meningoencephalitis is a rare manifestation, as the vast majority of affected patients have identifiable disease at another location, most commonly the lungs, skin or genitourinary tract.<sup>52</sup> Rarely, patients will present without evidence of disease elsewhere;<sup>52,55</sup> this is more likely to occur in individuals with intracranial abscesses than in those with meningoencephalitis.

In immunocompromised patients with blastomycosis, the central nervous system is more likely to be affected. Central nervous system involvement may occur in up to 40% of patients with the acquired immunodeficiency syndrome (AIDS) who acquire blastomycosis.<sup>60</sup> In one series of 15 patients with AIDS, six had CNS disease; four of these had meningitis and two had intracerebral abscesses. Despite the fact that most of the patients presented with obvious multisystemic disease, antemortem diagnosis of CNS involvement was made in only one-half of the cases.<sup>60</sup> Up to 10% of patients with other immunocompromising illnesses who acquire blastomycosis may develop CNS disease, and have included patients with sarcoidosis, rheumatic disorders, multiple myeloma and organ transplantation.<sup>61</sup> Chronic steroid therapy was the common factor predisposing to infection in these cases.

Diagnosis of CNS blastomycosis may be straightforward when other systemic sites are involved. Central nervous system radiographic studies are non-specific. MRI scans may mimic tuberculous meningitis with nodular meningeal enhancement. Lumbar puncture will demonstrate a typical picture of a chronic meningitis syndrome with lymphocytic pleocytosis, although occasional cases may have up to 40% polymorphonuclear leukocytes.<sup>62</sup> In the absence of an immunocompromising illness, stains of the CSF will almost uniformly be negative. In one series of 22 patients with CNS blastomycosis, only two patients had a positive culture from lumbar fluid,<sup>52</sup> while ventricular fluid cultures were positive in six of seven patients sampled.<sup>55,59</sup> The yield of culture has been said to be enhanced if larger volumes, e.g., 30–50 ml, are cultured. On occasion, meningeal biopsy, brain biopsy or posterior fossa exploration may be necessary to make a definitive diagnosis.<sup>52</sup> In patients with AIDS and other immunosuppressive illnesses, the yield of smears and cultures

is much higher.<sup>60,61</sup> Cerebrospinal fluid serologic studies have been of little assistance in the diagnosis of CNS blastomycosis.

Guidelines for the therapy of blastomycosis, including CNS disease, have been published; however, the recommendations regarding therapy of CNS disease have been based mostly on expert opinion, as no clinical trials have been performed given the relative infrequency of this particular disease manifestation.<sup>63</sup> Amphotericin B at a dosage of 0.7–1.0 mg/kg daily for a total dose of at least 2 g is the recommended regimen. Lipid preparations have not been studied but, based on experience with other CNS mycoses, are a reasonable option for individuals who cannot tolerate amphotericin B deoxycholate. Although itraconazole is the drug of choice for mild to moderate blastomycosis at other sites, azoles should not be considered for primary treatment of patients with CNS disease. In special circumstances, because of its favorable CSF pharmacokinetics, fluconazole could be considered at a higher dose, e.g., 800 mg daily.<sup>63</sup> Recently, there have been reports of several patients successfully treated with voriconazole after having relapsed on itraconazole therapy for CNS disease.<sup>64,65</sup>

Indications for surgical management of CNS infection due to blastomycosis include the need for a diagnostic biopsy of a mass lesion or undiagnosed meningeal disease and for treatment of mass lesions causing neurologic deficits, particularly when disease is located in the posterior fossa or spinal canal.<sup>66</sup>

## Coccidioidomycosis

*Coccidioides immitis* and *C. posadasii*, the etiologic agents of coccidioidomycosis, are relatively common causes of chronic meningitis in endemic regions. Disease is acquired by inhalation of infectious arthroconidia, followed by a pneumonitis. Most patients are asymptomatic at this stage; however, in approximately 0.5% the disease will disseminate outside the respiratory tract, and one-third of these will involve the CNS.<sup>67</sup> Certain patient populations are more likely to develop disseminated disease, including dark-skinned races, pregnant women, patients treated with corticosteroids, particularly solid organ transplant recipients, and up to 30% of patients with HIV infection;<sup>68</sup> this predisposition holds true for CNS disease, as well.<sup>69</sup> The VA-Armed Forces Cooperative Study of Coccidioidomycosis reviewed the records of 699 individuals with coccidioidomycosis, 25 of whom had CNS disease and records sufficiently complete to make conclusions.<sup>70</sup> In these patients, there was adequate information available to determine the time of exposure; seven of the 25 had onset of CNS illness less than 1 month after initial infection, nine had onset between 1 and 6 months and five had onset between 1.5 and 12.5 years. In another series of cases, the interval between primary infection and the onset of meningeal disease was less than 3 months in 62.5% of patients.<sup>69</sup>

Central nervous system involvement by *Coccidioides* can take several forms:

- subacute or chronic meningitis, which is the most common presentation
- encephalitis
- parenchymal microscopic granulomas
- abscesses
- vascular occlusion with infarcts
- radiculitis.<sup>71</sup>

Subacute or chronic meningitis is the most common manifestation of CNS disease due to *Coccidioides*.<sup>69-71</sup> It occurs early in the disease process and is quite unusual after 2 years.<sup>69</sup> Constitutional symptoms such as fever and weight loss are common and progress over 1–3 weeks. Headache was seen in one series in 23 of 31 patients and was described as throbbing, bilaterally intense and frequently accompanied by nausea and vomiting. Four patients had clinical indicators of intracranial hypertension including headache, vomiting, and papilledema.<sup>69</sup> More than one-half of the patients will have disorientation, lethargy, confusion or loss of memory. One-third may have meningismus. Focal neurologic signs are also seen frequently, including hyperreflexia, cranial nerve palsies, diplopia, and nystagmus.<sup>72</sup>

Encephalitis is almost as common as meningitis and probably results from extension of the inflammatory meningeal process along Virchow–Robin spaces across the pia into the underlying brain parenchyma.<sup>71</sup> Parenchymal microabscesses may also result from this process.

In patients with HIV infection, the extrapulmonary manifestations occur much more commonly. In one report of 77 patients, nine had meningitis.<sup>68</sup> CSF parameters in meningitis were similar to those seen in non-immunocompromised hosts with lymphocytic pleocytosis, hypoglycorrhachia, and elevated complement fixation antibody titers; CSF cultures, however, were more commonly positive (5 of 9). The CSF parameters were distinctly different from what is normally seen in patients with cryptococcal meningitis and AIDS, where the CSF cell count may be quite normal.<sup>68</sup>

Parenchymal brain involvement occurs in 1–33% of patients with CNS *Coccidioides*.<sup>73</sup> Because of the anatomic location of these lesions, they appear to arise from hematogenous spread; virtually all patients have foci of infection elsewhere, usually the lungs. Two-thirds of patients do have associated meningitis. Lesions may be single or multiple, they can be superficial or deep, and are found throughout the brain, although cerebellar involvement is frequent. The spinal cord may be involved.<sup>73</sup>

Chest radiographs may be abnormal on admission in up to 89% of patients.<sup>69</sup> Focal brain lesions due to *Coccidioides* may demonstrate contrast-enhancing masses by CT or MRI scanning.<sup>73</sup> Fifteen to 20% of patients will have evidence of vasculitic infarction.<sup>74</sup>

Approximately one-third of patients will have a moderate leukocytosis of greater than 10,000/mm<sup>3</sup>. In some series, peripheral eosinophilia of greater than 400 cells/mm<sup>3</sup> has been present in up to 19% of patients; however, this may correlate more strongly with disseminated disease than meningitis, per se.<sup>75</sup>

Examination of the CSF is usually consistent with a chronic meningitis; normal CSF findings do not exclude the diagnosis of *Coccidioides* meningitis.<sup>69</sup> Lumbar puncture has demonstrated an opening pressure >250 mmH<sub>2</sub>O in 30%. A lymphocytic pleocytosis is commonly found, with a mean CSF white blood cell count of 260/mm<sup>3</sup>. Eosinophilic pleocytosis may suggest the diagnosis. Ragland<sup>75</sup> found 70% of patients had eosinophilic pleocytosis with one-third actually having an eosinophilic meningitis as defined by >10 eosinophils/mm<sup>3</sup>; only 30% of his patients lacked eosinophilia in the CSF. Others have also commented on the presence of eosinophilia.<sup>70,76</sup> Hypoglycorrhachia with glucose <40 mg/dl occurred in 60%.

Protein was elevated in most with a mean of 165 mg/dl. CSF culture was positive in only about one-third of patients.<sup>69,70</sup>

Ventricular fluid differs substantially from lumbar CSF and is not suitable to rule out the diagnosis of *Coccidioides* meningitis, nor is it adequate to follow the effectiveness of therapy; ventricular CSF tends to have fewer cells, lower protein and higher glucose concentrations and a lower CF titer than that obtained from the lumbar space.<sup>69,77</sup>

Cerebrospinal fluid complement fixation titers to examine for IgG antibodies should be measured in all patients in whom the diagnosis of *Coccidioides* meningitis is being considered. The sensitivity of the test is greater than 80–85% and specificity is close to 100%. Titers range from 1:2 to 1:256. Patients with focal lesions may have negative complement fixation titers.<sup>73</sup>

Previously, the major problem in treatment of coccidioidal meningitis was the need for intrathecal amphotericin B, which had to be given over a long period with all its attendant problems, risks, and complications. Intravenous amphotericin B therapy alone is not adequate for treatment of CNS disease. Guidelines published by the Infectious Diseases Society of America now recommend oral azole therapy, specifically fluconazole.<sup>78</sup> Dosages of 400 mg/day were reported in clinical trials<sup>79</sup> but many authorities are now using 800–1000 mg/day based on the safety of these larger doses and relatively low cost of fluconazole. Itraconazole in doses of 400–600 mg/day has been shown to be equally effective as fluconazole and is an option.<sup>80</sup> Azole therapy should be continued indefinitely, as cure likely never occurs.<sup>81</sup> Recently, several reports have described successful treatment of *Coccidioides* meningitis with the use of voriconazole, which has the advantages of good in vitro activity, favorable pharmacokinetics when taken orally and good CNS penetration.<sup>82-84</sup> Another patient has been described who had meningitis refractory to conventional therapy and had a partial response to oral posaconazole; complete details of this case were not provided.<sup>85</sup>

Some clinicians use intrathecal (via hyperbaric lumbar or intracisternal injection) therapy initially to expedite response and for life-threatening disease.<sup>86</sup> Cisternal injections may be complicated by bleeding and intrathecal amphotericin B commonly causes a chemical arachnoiditis.<sup>86</sup> The use of Ommaya reservoirs has been tempered by the major drawbacks of infection and obstruction. Furthermore, the use of an Ommaya reservoir to deliver amphotericin B to the ventricles is not likely to benefit therapy as disease is not usually present in the ventricular location.

Optimal management of focal lesions has not been specifically investigated; however, satisfactory responses have been obtained with oral fluconazole alone.<sup>73</sup>

The adequacy of therapy for CNS disease should be monitored by improvement in symptoms and periodic assessment of CSF *Coccidioides* complement fixation titers which should decline to undetectable levels. CSF white blood cell counts may improve even in the absence of therapy.<sup>70</sup>

Communicating hydrocephalus remains an extremely common complication, even in patients who have had favorable responses to therapy, frequently occurring after many years of seemingly adequate therapy.<sup>69,75,87</sup> In Bouza's series of 31 patients with CNS *Coccidioides*, 15 patients ultimately developed hydrocephalus at some point.<sup>69</sup> In Ragland's series of 27 patients, nine developed hydrocephalus, eight of whom required a ventriculoperitoneal shunt for management.<sup>75</sup>

The most common life-threatening complication of *Coccidioides* meningitis is CNS vasculitis that can lead to cerebral ischemia, infarction, and hemorrhage.<sup>88</sup> In Williams' report of 10 cases, the onset of vasculitic or encephalitic complications was less than 1 month after onset of the original illness in five patients; 1–2 months afterwards in three; and greater than 2 months in two, one of which occurred 4 years after starting therapy for coccidioid meningitis.<sup>88</sup> Inflammatory reactions were present involving the walls of small- and medium-sized vessels and the adjacent perivascular zones. Parenchymal necrosis due to endarteritis obliterans was described. Neurologic manifestations occur abruptly without warning and lead to serious neurologic sequelae including hemiparesis, cranial nerve palsies, and altered states of consciousness or death. Optimal therapy for this devastating complication is not well defined, with some authorities advocating aggressive antifungal therapy that includes combined intrathecal and intravenous amphotericin B; others recommend the addition of corticosteroids, although the potential of aggravating the underlying infection is a real concern.<sup>74,78,88</sup>

## Cryptococcosis

*Cryptococcus neoformans* is the most frequent cause of fungal meningitis.<sup>89</sup> *C. neoformans* has two serotypes defined according to the antigenic specificity of the capsular polysaccharides: serotype A is ubiquitous, and serotype D is found in Europe with a heterogeneous distribution.<sup>90</sup> *Cryptococcus gattii* also has two serotypes: B and C. B serotype has been found in the vicinity of *Eucalyptus* trees (*E. camaldulensis*, *E. tericormis*) in Australia and Southern California.<sup>91,92</sup> Other species of trees, as well as the soil, have been implicated in recent human and animal outbreaks of *C. gattii* infections on Vancouver Island, British Columbia;<sup>93</sup> a similar *C. gattii* genotype has also been isolated from one patient in Washington State, indicating some potential geographic migration of the fungus.<sup>94</sup> The ecologic niche of C serotype is unknown. Serotypes A and B are present in pigeon and other bird droppings and in the earth. Only serotypes A, B, and D have been regularly isolated from diseases in humans or animals. In most cases, *C. neoformans* is responsible for a chronic meningitis affecting predominantly the basal meninges. *C. gattii* serotype B is rare in AIDS patients, even in areas where this serotype is present in non-AIDS patients.

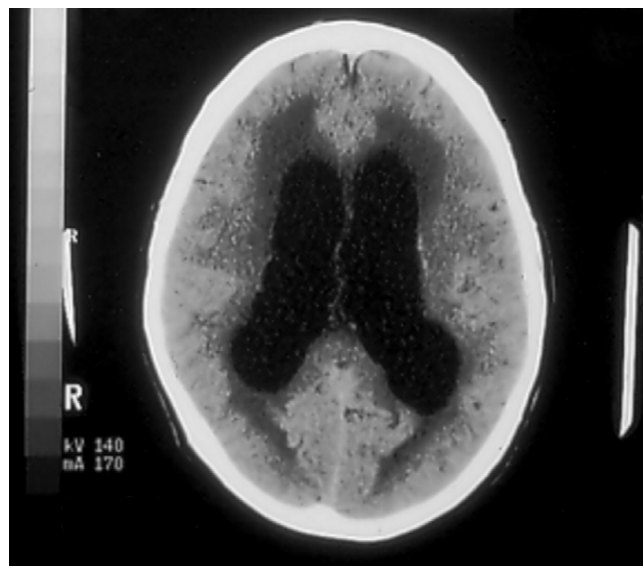
AIDS is now the leading predisposing factor for cryptococcal infection, illustrating the importance of cellular immunity as a mechanism of protection. Primary infection is generally pulmonary and is acquired by inhalation of yeasts present in the environment. Hematogenous dissemination can occur during this initial infection. The fungus has a marked tropism for meninges. Meningitis is observed during or several years after the primary infection. Hodgkin's disease, lymphoma, sarcoidosis, and idiopathic CD4+ lymphocytopenia are other conditions incurring a predisposition to cryptococcosis.<sup>95–97</sup> Corticosteroids, treatment for solid organ transplantation, and therapy with biologic response modifying agents (e.g., infliximab) also predispose a person to this infection.<sup>98</sup> Before the AIDS epidemic, 30–40% of patients with cryptococcosis apparently had no immune deficit, although it is likely that careful searches for subtle immune deficits have not been regularly done.

In a retrospective study of 133 cases of cryptococcosis in Victoria, Australia, all *C. gattii* infections occurred in healthy hosts, and 90% of *C. neoformans* occurred in immunosuppressed hosts.<sup>99</sup> None of the 20 patients with *C. gattii* died; however, they often experienced neurologic sequelae that required surgery and prolonged antifungal therapy. Meningitis was the most common manifestation for both varieties but focal CNS (7 of 20 patients) and pulmonary (11 of 20 patients) lesions occurred primarily in healthy hosts infected with *C. gattii*.

Brain mass lesions from *C. neoformans* are much less common than meningitis for serotypes A and D. On the contrary, serotype B, frequent in apparently immunocompetent patients, often produces a pseudotumor mass in the lung and in the brain. In rare instances, mass lesions can be present without meningitis.<sup>100</sup> The lesions are situated in Virchow–Robin space or in the brain tissue. These cryptococcoma or cryptococcal granulomatous lesions are either gelatinous cysts or solid granulomatous lesions. The course of the disease is often subacute.

In some rare cases of cryptococcal meningitis, the onset of disease is acute over a few days with fever, headache, vomiting, and nuchal rigidity suggestive of meningitis. In most cases, the disease has a mild to chronic course with prolonged fever or headache or both for weeks before the occurrence of nausea or vomiting or some degree of obtundation or cranial nerve palsy, usually due to increased intracranial pressure. In patients with AIDS and CD4 lymphocyte counts less than 100/mm<sup>3</sup>, unexplained fever may be the only initial symptom. On occasion, meningitis can be asymptomatic or present with findings consistent with normal pressure hydrocephalus (Fig. 28-1).<sup>101–106</sup>

Of all the fungal etiologies of meningitis, *Cryptococcus* is one of the easiest diagnoses to make. Blood, sputum, and urine cultures should be performed. Routine blood cultures are positive about half the time in patients with HIV infection, usually growing within 2–3 days. Serum cryptococcal antigen titers are specific and sensitive (>96%) and can be used to screen for



**Figure 28-1** Radiographic demonstration of hydrocephalus in a patient with cryptococcal meningitis that presented with a syndrome consistent with normal-pressure hydrocephalus.



the disease in patients with HIV infection.<sup>101</sup> Lumbar puncture is the diagnostic procedure of choice. Opening pressure, which sometimes can be extremely high, should always be measured to assist with prognosis and specific therapeutic interventions. CSF is usually clear, and cellular reaction and biochemical alterations can be minimal or absent in patients with severe cellular immunodeficiency. In patients without HIV infection, the CSF profile shows a lymphocytic pleocytosis with elevated protein and hypoglycorrhachia. The presence of *C. neoformans* by direct examination with India ink or Gram stain and culture confirms the diagnosis. Detection of cryptococcal polysaccharide antigen in CSF or serum is highly specific and sensitive,<sup>101-105</sup> although on rare occasions the test may be negative when infection is caused by a capsule-deficient strain.<sup>107</sup>

Guidelines for the management of cryptococcal meningitis have been published and are based, to some extent, on randomized, controlled clinical trials.<sup>108</sup> For patients who are not HIV infected, recommended therapy includes amphotericin B 0.7–1.0 mg/kg/day along with 5-flucytosine 100 mg/kg/day for 2 weeks followed by oral fluconazole 400 mg/day for a minimum of 10 weeks. With this therapy, sterilization of the CSF can be expected in 60–90% of patients after 2 weeks. An alternative regimen consists of amphotericin B 0.7 mg/kg/day plus 5-flucytosine 100 mg/kg/day for 6–10 weeks without subsequent therapy with fluconazole. For HIV-infected patients, the same initial regimen is suggested.<sup>108,109</sup> For both groups of patients, a lipid preparation of amphotericin B can be substituted for amphotericin B in individuals in whom impaired renal function is a concern. At the end of 2 weeks of therapy for all patients, it is prudent to perform a lumbar puncture to assess the efficacy of therapy; continued positive cultures support the provision of longer courses of intravenous therapy.

Monotherapy with fluconazole has been suggested as an option in individuals who have normal neurologic function and cryptococcal antigen titers less than 1:1024, and is regularly provided to patients in resource-limited countries.<sup>108,110-112</sup> However, this therapy is fraught with problems, including inadequate fungicidal activity resulting in delayed clearance of the organisms from the CSF as well as the real potential for development of resistant isolates.<sup>107,108</sup> Preliminary evidence suggests a possible role for adjunctive therapy with recombinant interferon- $\gamma$ 1b. A study in patients with HIV-related acute cryptococcal meningitis demonstrated no adverse effects on CD4 cell counts or HIV viral load and trends towards improved mycologic and clinical success in interferon recipients.<sup>113</sup> It was suggested that augmentation of the host immune response may have a role in treatment of invasive fungal infections, particularly in those cases where the therapeutic response is not optimal.

Following 10 weeks of therapy, patients with HIV infection should be provided maintenance therapy with fluconazole at a dose of 200 mg/day, as the relapse rate is in excess of 50% in the absence of improvement of the CD4 cell count.<sup>114</sup> Maintenance therapy can be safely discontinued in patients who have sterilization of the CSF, and have been receiving highly active antiretroviral therapy (HAART) if their CD4 cell count has increased to  $>100$  cells/mm<sup>3</sup> and there was an undetectable HIV RNA level that had been sustained for more than 3 months.<sup>115</sup>

High intracranial pressures have been associated with a poor prognosis.<sup>116</sup> Guidelines have been promulgated to

manage the increased intracranial pressures, which include aggressive use of large-volume lumbar punctures, lumbar drains or ventriculoperitoneal shunting as necessary to keep intracranial pressures below 250 mmH<sub>2</sub>O.<sup>108,116</sup> Adjunctive corticosteroid therapy has been discouraged<sup>108</sup> and the use of acetazolamide has been associated with significant depressions of bicarbonate levels and more frequent adverse reactions.<sup>117</sup> Major departures from these published guidelines are common in clinical practice, resulting in failure to adequately control the increased intracranial pressures with the subsequent development of clinically significant neurologic injuries.<sup>118</sup>

Although the serum cryptococcal antigen can be valuable in the initial diagnosis of cryptococcal meningitis, the utility of serial determinations to follow the effectiveness of therapy has not been demonstrated. Even though in the majority of patients the cryptococcal antigen titers appear to decrease over time, there is not a significant correlation between the titer results of patients who had clinical responses to treatment and those who experienced persistent disease or relapse.<sup>119</sup>

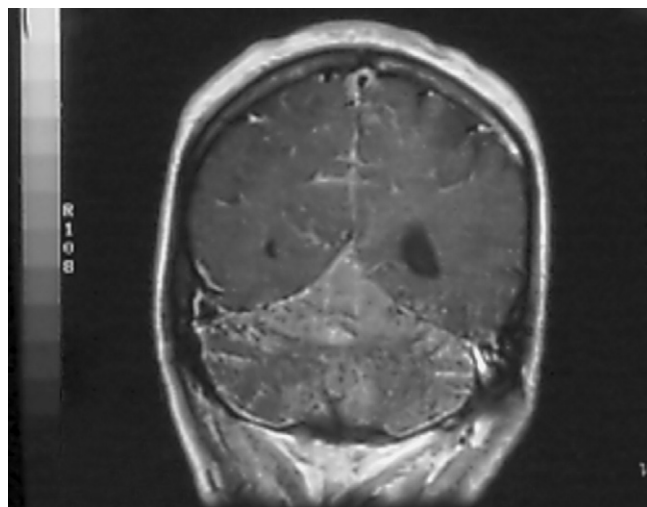
Approximately one-third of all HIV-infected patients hospitalized with cryptococcal meningitis who received HAART will develop a paradoxical worsening of symptoms attributed to an exaggerated inflammatory response, the so-called immune reconstitution inflammatory syndrome (IRIS).<sup>120-122</sup> Patients are more likely to develop this syndrome if they are naive to antiretroviral therapy, have advanced HIV disease or initiate HAART in close proximity to the acute meningitis episode, and have rapid declines in the HIV viral load.<sup>120-122</sup> Differentiation of the immune reconstitution inflammatory syndrome from symptomatic relapse of the cryptococcal disease can be difficult; patients with *C. neoformans*-related IRIS tend to have higher CSF opening pressures, glucose levels and WBC counts compared with patients who have symptomatic relapse of meningitis.<sup>121</sup> CSF cultures tend to be sterile; however, patients can develop IRIS and still have positive CSF cultures.<sup>112</sup> Imaging studies may show meningeal enhancement, even in patients whose studies were relatively normal during the acute episodes (Fig. 28-2). Effective management of IRIS includes aggressive measures to control the elevated intracranial pressures, continuation of both antifungal and antiretroviral therapy as well as the judicious use of antiinflammatory agents, such as corticosteroids.

## Histoplasmosis

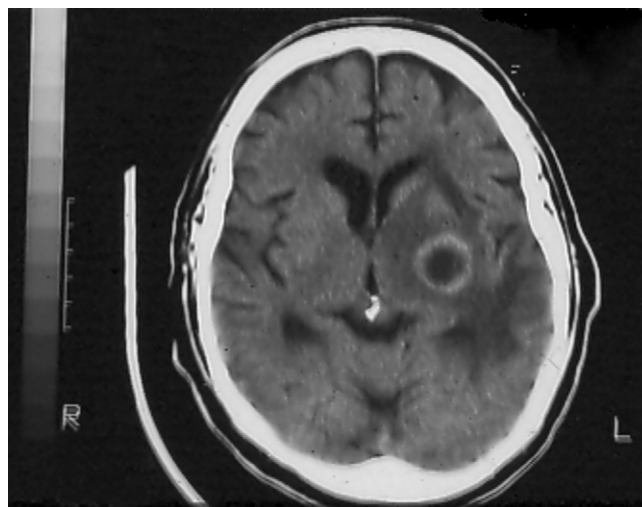
Approximately 10–20% of patients with progressive disseminated histoplasmosis have clinically apparent CNS system involvement but not all patients with CNS infection have symptomatic disease at other sites.<sup>123</sup> Disease can occur as a result of reactivation as evidenced by infection diagnosed in non-endemic regions.

A number of manifestations may accompany CNS histoplasmosis. Parenchymal mass lesions of the brain or spinal cord may account for 24–39% of cases of CNS disease, and occur more commonly with *Histoplasma* than most other mycoses that involve the CNS. They may be single or multiple (Fig. 28-3). Patients typically present with headache, focal neurologic deficits, and altered mentation.<sup>124</sup>

Up to 16% of the time, patients with CNS disease will have diffuse encephalitis as a manifestation. Meningitis is the



**Figure 28-2** Enhancement of cerebellum in a patient with AIDS who presented with blindness as a result of *Cryptococcus*-associated immune reconstitution inflammatory syndrome.



**Figure 28-3** Solitary histoplasma in an HIV-negative patient.

manifestation in up to 40% of patients. It may accompany overt episodes of disseminated disease or, in 25% of cases, may present as isolated chronic meningitis without evidence of disease elsewhere. Patients who have been treated successfully for disseminated histoplasmosis may occasionally relapse in the CNS with meningitis. The clinical presentation may differ somewhat between immunocompetent and immunocompromised patients. Those patients without obvious defects in immunity tend to have signs indicative of an inflammatory process, e.g., headache, meningeal irritation, focal neurologic signs, and ataxia. Mental status changes and cranial nerve abnormalities appear to occur equally often. Fever, on the other hand, may occur more often in immunocompromised patients, probably because of more extensive dissemination of disease.<sup>123-125</sup> Hydrocephalus is common.<sup>125</sup>

Other unusual presentations include symptomatic cerebral emboli associated with infective endocarditis and stroke syndromes, likely due to a vasculitis involving the vasculature at the base of the brain.

In patients with idiopathic hydrocephalus, *Histoplasma* infection has caused ventriculoperitoneal shunt malfunction in the absence of typical causes, like bacterial infection.<sup>126,127</sup>

Diagnosis of CNS *Histoplasma* infection has not been as difficult as some of the other mycoses because of the availability of various testing modalities. Analysis of the CSF demonstrates values consistent with a chronic meningitis, with a lymphocytic pleocytosis in 90%, elevated protein in 80%, and hypoglycorrhachia in 82%.<sup>123</sup> Sensitivity of cultures of CSF varies between 27% and 65%, although cases have been reported in which up to 10 different lumbar fluid samples had to be sent in order to successfully culture the organism.<sup>123,128</sup> It is recommended that at least 10 ml of CSF should be cultured to improve the yield.<sup>128</sup> The sensitivity of the CSF *Histoplasma* antigen test varies between 38% and 67%, and has a high specificity of 96%; in the case of CNS infection, antigen levels in the CSF may be higher than serum or urine levels. Patients with HIV infection are more likely to be positive and

patients not infected with HIV will be on the lower end of positivity.<sup>123,129</sup> Serologic tests for anti-*Histoplasma* antibodies in the CSF may assist diagnosis, having positive results in up to 80% of cases.<sup>123,130</sup> Cultures of blood and bone marrow may also facilitate diagnosis.

Guidelines for the treatment of histoplasmosis have been published recently.<sup>131</sup> No comparative studies for the treatment of CNS disease have been performed, so the recommendations promulgated in the guidelines are based on small series of patients and expert opinion. The recommended regimen for treatment of CNS disease is liposomal amphotericin B 5.0 mg/kg daily for a total dose of 175 mg/kg given over 4–6 weeks, followed by itraconazole at a dosage of 200 mg two or three times daily for at least 1 year and until resolution of CSF abnormalities. It was suggested that CSF *Histoplasma* antigen levels be monitored to demonstrate clearance. In addition, blood levels of itraconazole should be obtained after about 2 weeks of therapy to ensure adequate drug exposure.<sup>128,131</sup>

Cerebrospinal fluid analysis should be performed after the second week of therapy if the patient has not shown a clinical response. Otherwise, another analysis should be performed after the first month of therapy, when amphotericin B is replaced by azole therapy, if relapse is suspected on clinical grounds, and after 1 year of therapy when a decision regarding discontinuation of therapy is to be made. Persistence of a cellular response or failure to clear *Histoplasma* antigen is sufficient evidence to support continuation of therapy.<sup>128</sup>

Data from animal models have raised concerns about the adequacy of brain tissue levels of itraconazole because of evidence of P-glycoprotein mediated transport of itraconazole across the blood–brain barrier out of the CNS.<sup>132</sup> However, itraconazole has been shown to be effective in the treatment of CNS infections, including multiple cerebral histoplasmoses.<sup>133</sup>

Salvage treatment of a case of *Histoplasma* meningitis refractory to multiple other antifungals, but successfully treated with oral posaconazole 400 mg twice daily, has been reported.<sup>134</sup>

## Paracoccidioidomycosis

*Paracoccidioides brasiliensis* is endemic to subtropical areas of Central and South America with most cases occurring in Brazil, Colombia, and Venezuela. Disease has been described in patients who have left endemic areas, indicating the potential for late reactivation. There is a marked male predominance of the disease.<sup>135,136</sup> In non-CNS infection, the male:female ratio of disease is 9:1; with CNS disease, the ratio is as high as 23:1. Exposure does not account for these differences, however, as serologic positivity is equivalent between the sexes in endemic regions. Inhibitory effects of estrogens on transition of conidia to yeast forms likely account for these differences between the sexes.<sup>137</sup> The mean age in several descriptions of patients with CNS disease has been 44 years. In one large report of 173 cases, 24 had CNS involvement for an incidence of 13.9%, although it has been as high as 25.4% in other series.<sup>136</sup>

The lung is the primary site of infection with *P. brasiliensis* and is frequently asymptomatic. Lymphohematogenous spread may occur subsequently at any time, resulting in chronic infection. The CNS can be the sole site of dissemination with no evidence of disease elsewhere,<sup>135</sup> although most commonly there is evident disease at other locations, including the lungs (80%), skin (35%) and oropharyngeal mucosal surfaces (20%). In one report, neurologic symptoms appeared before systemic symptoms in 21%, concurrent with systemic symptoms in 33% and after systemic symptoms in 46%.<sup>136</sup>

In almost all cases, *P. brasiliensis* CNS disease presents with parenchymal involvement, which is frequently multifocal; a small proportion have associated meningoencephalitis. Seizures are the most common presenting symptom and occur in about one-third of cases, along with hemiparesis (25%), cerebellar signs (25%), and headache (21%).<sup>136</sup>

Diagnosis of CNS paracoccidioidomycosis may be difficult because of the non-specificity of the symptoms, low sensitivity of presently available tests, and the fact that the most common form of disease is isolated to the CNS, and occurs in the absence of evidence of systemic infection. Computed tomography may show four radiographic patterns of disease:<sup>138</sup> approximately 45% of patients will demonstrate low-density lesions with ring enhancement; 20% will have some calcification with ring enhancement; 10% will show multiloculated low-density lesions with ring enhancement; and 5% will have diffuse subarachnoid enhancement. Fifty percent of cases have multiple masses. The cerebral hemispheres are affected in 35%, cerebellum in 35%, and brainstem in 15%. The spinal cord is affected in about 5%, with the thoracic (58%) and cervical (25%) portions of the cord being the most commonly involved locations. Only about 10% have subarachnoid space disease demonstrable radiographically. Evidence of hydrocephalus will be present in approximately 30%.<sup>138</sup>

Spinal fluid analysis is not usually helpful in making the diagnosis as it frequently shows relatively minor abnormalities of the cell count (3–14 cells/mm<sup>3</sup>), normal glucose, and normal or mildly elevated protein. Microscopic examination of the cerebrospinal fluid is uniformly negative for fungus and culture is rarely positive. Routine double immunodiffusion serologic testing of the CSF is negative in almost all occasions and does not facilitate diagnosis.<sup>136</sup>

Recent studies have reported promising results utilizing detection of antigen and antibody in the CSF to detect two glycoprotein components of the organism.<sup>139</sup> In one study, gp43 and gp70 antigens were detected in the CSF in all patients tested and in 91% of serum samples from these patients using an inhibition ELISA. Antigen titers were higher in the CSF than the serum. Levels of gp43 were always higher than gp70. A standard ELISA to detect antibodies to gp43 and gp70 was also as sensitive as, and much easier to perform than, the inhibition ELISA. The authors suggested that the standard ELISA for gp43 and gp70 should be routinely employed and the inhibition ELISA reserved for cases in which there is high clinical suspicion but all other tests are negative.<sup>140</sup>

In most cases, the response to therapy is favorable. Trimethoprim-sulfamethoxazole has been used most commonly because of ease of administration of the oral medication, low cost and high CSF levels. In one series of 24 patients with CNS disease, four patients died and 20 had a good response using a dose of 2400 mg sulfamethoxazole and 480 mg trimethoprim daily administered in three divided doses.<sup>136</sup> There have been reports of successful therapy with both itraconazole and voriconazole.<sup>141,142</sup>

Neurosurgery is indicated for: patients with masses that do not respond to therapy; intracranial hypertension related to hydrocephalus, which may complicate up to 12% of cases; signs or symptoms of spinal cord compressions; and assistance with diagnosis if there is no peripheral evidence of disease.<sup>138</sup>

## Sporotrichosis

Central nervous system infection due to *Sporothrix schenckii* is extremely rare. Risk factors that appear to be important for development of CNS disease include alcoholism<sup>143</sup> and the acquired immunodeficiency syndrome.<sup>144–147</sup> A patient on immunosuppressive therapy for renal transplantation has been described with recurrent *Sporothrix* meningitis.<sup>148</sup> Features of CNS disease due to *Sporothrix* are characteristic of chronic meningitis involving the basilar meninges, with headache being present in almost all cases along with seizures, back pain, gait disturbances, ataxia, confusion, and fever.<sup>143,149–151</sup> Patients with HIV infection and sporotrichosis present with widespread disease, especially multiple cutaneous lesions. Meningoencephalitis and hydrocephalus have been regular features of HIV-related illness.<sup>144–147</sup>

In most reported cases, the delay in diagnosis of *Sporothrix* CNS disease has been considerable, because of failure to consider the diagnosis and send appropriate specimens, failure to appreciate the significance of a cultured isolate, or inability to grow the organism because of its typical low numbers in CSF. In one series, the time from onset of symptoms to the first positive culture results varied from 3 to 11 months, with an average delay of 6.5 months.<sup>151</sup> In only one case was there evidence of *Sporothrix* infection elsewhere that would have aided diagnosis. Lumbar puncture is usually characterized by normal opening pressures, lymphocytic pleocytosis (70–99% in most cases), elevation of protein (190–808 mg/dl), and hypoglycorrhachia (9–26 mg/dl). Cultures are regularly negative, although it has been suggested that yield may be improved by performing multiple large-volume lumbar punctures or utilizing cisternal punctures along with membrane filtration culture techniques.<sup>143</sup>



It needs to be emphasized that *S. schenckii* should never be considered a laboratory contaminant and isolation should prompt institution of appropriate therapy.<sup>143,149,152</sup> In cases where a diagnosis of CNS disease has been made, it was facilitated by a positive CSF antibody to *Sporothrix* by enzyme immunoassay (more sensitive) or latex agglutination (less sensitive).<sup>151</sup> It has been recommended that antibody be measured in any patient with chronic meningitis for which no cause is discovered by usual diagnostic testing.<sup>151</sup>

Guidelines have recently been published for the management of disease due to *S. schenckii*, including that involving the CNS.<sup>153</sup> However, given the paucity of cases, the recommendations for therapy are largely empiric. Amphotericin B, given as a lipid preparation in a dosage of 5 mg/kg daily for 4–6 weeks, is the recommended initial treatment. Because of its better safety profile, the authors preferred a lipid preparation over conventional amphotericin B deoxycholate, although this could be used at a dosage of 0.7–1.0 mg/kg daily. Itraconazole at a dosage of 200 mg twice daily is recommended as step-down therapy after a satisfactory therapeutic response has been achieved with an amphotericin B preparation. The itraconazole should be continued for at least 12 months of therapy. Because of concerns regarding the erratic pharmacokinetics of itraconazole, it was recommended that levels be obtained after 2 weeks of therapy to ensure adequate drug exposure. Cerebrospinal fluid antibody titers fall with successful treatment of the disease and closely parallel improvement in other CSF indices, including cell count, glucose, and protein. For individuals with AIDS and other immunocompromising illnesses, suppressive therapy with itraconazole at a dose of 200 mg daily is recommended to prevent relapse. It is not clear if the drug can be discontinued in patients with AIDS who develop a satisfactory response to highly active antiretroviral therapy.

## Zygomycosis

Several well-defined patient groups that develop CNS disease due to the various zygomycetes have been identified, and include:

- diabetics with ketoacidosis
- patients with acute leukemia receiving chemotherapy
- solid organ transplant recipients receiving immunosuppressive agents
- patients undergoing hematopoietic stem cell transplantation because of steroid use for graft-versus-host disease
- patients, usually on hemodialysis, receiving deferoxamine chelation therapy for iron overload conditions
- parenteral drug abusers.<sup>154</sup>

Rhinocerebral disease represents one-third to one-half of all cases of CNS zygomycosis, the majority of which occur in the setting of diabetic ketoacidosis.<sup>155</sup> In 929 cases of zygomycosis reviewed by Roden, 283 had CNS infection: 69% were rhinocerebral, 16% were localized cerebral, and 15% had hematogenous spread.<sup>156</sup> Mortality for cerebral and rhinocerebral disease was 62%. Disease in individuals without known risk factors is very rare.<sup>157–159</sup>

Rhinocerebral disease occurs by way of direct extension of sinus disease into the orbit, eye, optic nerve, and brain

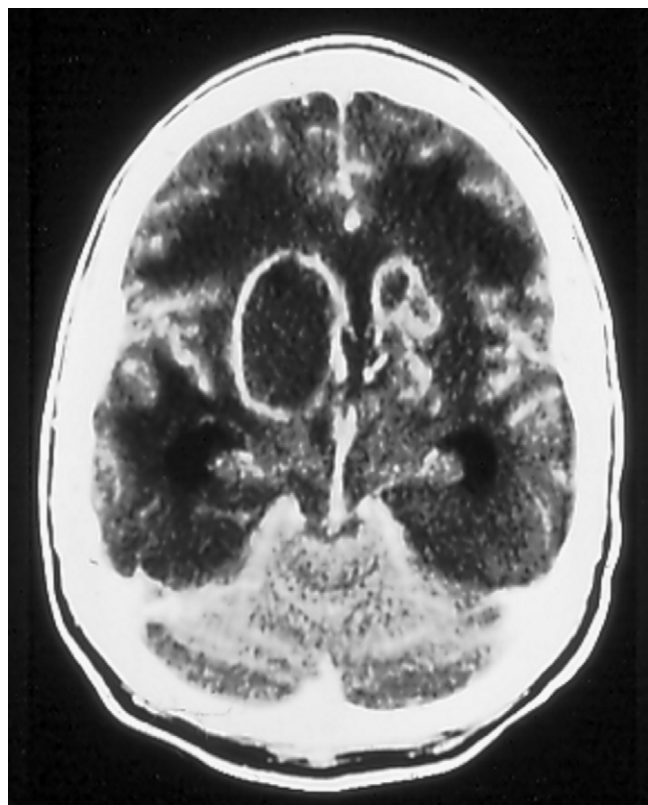


**Figure 28-4** Bilateral frontal lobe abscesses due to *Rhizopus* sp. in a patient with diabetic ketoacidosis.

parenchyma, including the frontal and temporal lobes (Fig. 28-4). Because of the angiotropism of the zygomycetes, patients may develop vascular manifestations such as: cavernous sinus thrombosis; isolated ischemic infarction secondary to internal carotid or basilar arteritis; and hemorrhagic infarction due to angioinvasion with formation and rupture of mycotic aneurysms.<sup>160</sup>

Verma recently reviewed the literature on isolated cerebral zygomycosis and found 31 cases.<sup>157</sup> Only three had no predisposing factor for zygomycosis; these presented with focal cerebritis,<sup>157</sup> meningitis,<sup>158</sup> and hydrocephalus.<sup>159</sup> Of the remaining 28 cases, 17 were intravenous drug abusers, four had acute lymphocytic leukemia and two each had diabetes mellitus and hepatic cirrhosis. In parenteral drug addicts with CNS zygomycosis, there is a predilection for the basal ganglia and thalamus (Fig. 28-5). Sixty-two percent of cases in parenteral drug addicts involve cerebral structures, whereas only 4% of patients in other risk groups have isolated cerebral involvement. It is likely that fungal conidia are inoculated with their drugs. Particulate matter the size of sporangioconidia tend to distribute to either the gray-white junction of the brain or the basal ganglia via the striatal arteries.<sup>156</sup>

Initial symptoms of rhinocerebral disease are consistent with either sinusitis or periorbital cellulitis and include eye or facial pain with numbness. Patients have headache, fever, and ocular pain. Facial edema is the earliest sign, with conjunctival suffusion, blurring of vision, and soft tissue swelling.<sup>154,161</sup> Patients may rapidly progress to develop internal carotid artery and cavernous sinus thrombosis.<sup>160</sup> On visual



**Figure 28-5** Characteristic basal ganglia abscesses due to zygomycosis in a parenteral drug addict.

inspection, infected tissues may appear normal initially but will then progress to an erythematous phase, then to a violaceous stage and then to development of a black, necrotic eschar as blood vessel thromboses and tissue infarction ensues.<sup>154</sup>

The diagnosis of invasive zygomycosis is frequently obvious from the clinical presentation; however, certain patient groups (e.g., parenteral drug addicts) may have presentations that make identifying a diagnosis challenging. Histologic demonstration of fungal invasion is necessary for a definitive diagnosis. All specimens should be cultured on appropriate fungal medium; however, it is not unusual for specimens that have shown typical organisms on histologic sections to be culture negative, particularly if tissue is ground before culturing.

Radiographic visualization may initially be normal. Cranial CT may show soft tissue swelling in paranasal sinuses and orbital contents with resultant exophthalmos. Some cases may exhibit cerebral infarction.

Despite aggressive surgical debridement and antifungal therapy, mortality from zygomycosis is at least 50%, and approaches 80–100% in patients with CNS disease.<sup>154</sup>

Several factors have been identified that are critical for successful outcomes and include the rapidity of diagnosis; reversal of the underlying predisposing factors, if possible; appropriate and early surgical debridement of infected tissues, with frequent reoperation as necessary; and appropriate antifungal therapy.<sup>154,162</sup> Amphotericin B and amphotericin B lipid preparations have been the most frequently utilized antifungal agents and, until recently, the only agents with demonstrated activity against the zygomycetes. Survival rates as high as 50%

have recently been described with a combination of aggressive surgery and amphotericin B-based therapy.<sup>154,163,164</sup>

Posaconazole has in vitro efficacy against some zygomycetes. When used as salvage therapy in compassionate-use protocols, there was a 72.7% response rate in 8/11 patients with cerebral disease.<sup>165,166</sup> Few details of specific cases were provided.

One patient has been reported who responded to treatment with an iron-chelating agent, deferasirox, after substantial progression of CNS disease following months of therapy with high-dose liposomal amphotericin B and caspofungin.<sup>167</sup> Deferoxamine chelator therapy has been considered a risk factor for the development of zygomycosis because of the organism's ability to specifically bind to the deferoxamine-iron complexes, strip the iron from the chelator through a reductive process, and facilitate iron uptake. However, animal models of zygomycosis have shown that deferasirox significantly improves survival and decreases tissue fungal burden by starving the zygomycetes of iron that is essential for growth and virulence.<sup>168</sup>

## Miscellaneous mycoses

### *Penicillium* Species

Infection due to *Penicillium marneffe* is restricted to Southeast Asia, where it is a major opportunistic infection, occurring in 17% of HIV-positive patients. CNS locations are rarely seen in disseminated cases. In a series of 92 cases, the fungus was isolated in three of 20 CSF specimens cultured and no brain lesions were noted.<sup>169</sup>

### *Scedosporium*

*Scedosporium apiospermum* (teleomorph *Pseudallescheria boydii*) has emerged among newly recognized pathogens. It has the same epidemiology as *Aspergillus* spp., occurring particularly in neutropenic patients and in patients treated with immunosuppressive agents including corticosteroids, azathioprine, cyclosporine, and tacrolimus.<sup>170-172</sup> Brain abscesses due to *S. apiospermum* have the same clinical presentation as those due to *Aspergillus* spp., with signs and symptoms suggestive of a space-occupying lesion, including fever, headache, and focal neurologic deficits.<sup>172</sup> *Scedosporium apiospermum* can cause pneumonia and brain abscess in near-drowning victims, because of its presence in polluted waters.<sup>173,174</sup> Patients develop manifestation of brain abscesses from a few days to several weeks after the near-drowning event.<sup>172</sup> *Scedosporium prolificans* is more often responsible for osteoarticular infections than CNS infection.<sup>175</sup>

The access to the brain can be hematogenous or spread from a sinus, or it can be due to trauma. Meningitis due to *Scedosporium* is rare and usually associated with immunosuppression, a near-drowning event, presence of a CSF drainage device or after intraspinal injections of anesthetics.<sup>172,176,177</sup> CSF examination shows elevated WBC and protein levels, and hypoglycorrhachia. Cultures are regularly negative.<sup>172</sup> CNS infections have also been described rarely in AIDS patients.<sup>178,179</sup> In vitro data, animal models, and recent experience in humans indicate that voriconazole is the primary drug of choice for treatment of pseudallescheriasis, sometimes in combination with

terbinafine.<sup>172-172,174</sup> In vitro data suggests some activity of posaconazole against *Scedosporium* spp.<sup>172</sup>

## Fusarium

*Fusarium* species are common in soil and water and are plant pathogens. Disease can be severe in neutropenic patients. The mechanism of infection is generally by inhalation into the lungs. Hematogenous dissemination with positive blood cultures and skin locations can occur and facilitate diagnosis. Infection of skin, nail, eye or catheter has been described and can lead to dissemination in severely immunosuppressed patients. *Fusarium* does not have a particular tropism for the CNS; a limited number of brain abscesses have been reported.<sup>180-184</sup>

## Dematiaceae Fungi

A group of filamentous fungi with dark-pigmented hyphae, which can cause severe infections. *Cladophialophora bantiana* is a well-known agent of cerebral infection in normal hosts.<sup>185-187</sup> Among several other genera, *Bipolaris* or *Exserohilum* (formerly *Drechslera* in part), *Curvularia*, *Fonsecaea*,<sup>186</sup> and *Wangiella* (*Exophiala*) often have been reported as a cause of infection in non-immunocompromised hosts.

*Cladophialophora bantiana* has a remarkable neurotropism. This fungus can be isolated from detritus. In many cases immunocompetent hosts are infected; often multiple abscesses are present, suggesting a hematogenous seeding of the brain. The lesion is usually located in the frontoparietal lobes and is either well demarcated or poorly circumscribed, the latter having a worse outcome. The course of disease is generally slow, allowing confirmed diagnosis by aspiration or surgical resection.

Revankar reviewed 101 cases of primary CNS phaeohyphomycosis; a total of 24 different species of fungi were isolated.<sup>187</sup> *Cladophialophora bantiana* accounted for 48 and caused brain abscesses in almost all of the cases. *Ramichloridium mackenzii* was seen in 13 cases, the next most frequent causative agent. *Ochroconis gallopavum* was the etiologic agent in five. Thirteen species were associated with only a single case report. More than half the patients had no underlying diseases. Thirty-seven patients had some sort of immunologic dysfunction, including malignancy in 10, neutropenia in four, bone marrow transplantation in three, solid organ transplantation in 15, injection drug use in six, and one with AIDS. Brain abscess was the most common presentation, seen in 87 cases; 67 had single lesions and the remainder had multiple lesions. Patients were treated with a combination of medical and surgical therapy. Amphotericin B was the most commonly prescribed antifungal agent in 59 patients but a variety of other drugs were used, most notably itraconazole. Overall mortality was 73%.<sup>187</sup>

## Other fungi

There are a number of anecdotal reports of a variety of rare species implicated in CNS infections: meningitis due to *Rhodotorula rubra*<sup>188,189</sup> or *Rhodotorula* spp.<sup>190</sup> or to *Blastoschizomyces capitatus*,<sup>191,192</sup> brain abscess due to *Wangiella dermatitidis*,<sup>193-195</sup> *Trichosporon beigeli*,<sup>196</sup> *Trichoderma longibrachiatum*,<sup>197</sup> *Chaetomium strumarium*,<sup>198</sup> *Chaetomium atrobrunneum*,<sup>199</sup>

*Schizophyllum commune*,<sup>200</sup> *Paecilomyces*,<sup>201</sup> *Penicillium* spp.,<sup>202-204</sup> *Metarrhizium anisoplia*,<sup>205</sup> *Microascus cinereus*,<sup>206</sup> *Curvularia clavata*,<sup>207</sup> *Ramichloridium obovoideum*,<sup>208</sup> and *Trichophyton* spp.<sup>209</sup>

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# Hematogenously disseminated fungal infections

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Although many of the invasive mycoses become apparent when they involve an obvious target organ such as the lung or brain, some can also present without localization (Table 29-1). The principal common manifestation of these forms of the invasive mycoses is fever. Widespread hematogenous dissemination may occur with almost any of the mycoses, and this is particularly evident among immunocompromised hosts. Unrecognized and untreated, these infections may result in serious sequelae, including death. Fortunately, the clinical settings in which these diseases are usually seen and the clinical and radiographic manifestations of dissemination often suggest the diagnosis of disseminated mycosis in these patients.

## Nosocomial fungi

### *Candida* spp.

The term “invasive candidiasis” encompasses a variety of conditions, including most commonly candidemia, acute disseminated candidiasis, renal candidiasis, endocarditis, meningitis, endophthalmitis, intraabdominal disease, and chronic hepatosplenic candidiasis, although virtually any organ can be involved. It typically occurs in hospitalized patients, with the most important hosts being intensive care unit (ICU) patients, neutropenic patients, transplant recipients, and neonates. Most normal hosts have easily identifiable risk factors such as prolonged ICU stay, intravenous catheters, hyperalimentation, diabetes, hemodialysis, recent surgery (particularly gastrointestinal (GI) surgery), or exposure to broad-spectrum antibiotics. The incidence of infection has been found to peak around day 10 of ICU stay.<sup>1</sup> *Candida* is now the fourth most common bloodstream isolate and the most common invasive fungal infection in critically ill non-neutropenic patients.<sup>2-4</sup> Diagnosis is difficult because often the only symptoms are fever and leukocytosis, with no other physical findings. Findings may be limited to fundoscopic examination, where the classic lesion is the cotton wool spot, a white retinal lesion(s) with vitreal extension, but this is seen infrequently.<sup>5</sup>

Diagnosis has traditionally depended on histopathology or blood cultures, which have relatively low sensitivity. The sensitivity of blood culture has improved with newer techniques such as lysis centrifugation, BACTEC high blood volume fungal media system, and the BacT/alert system.<sup>6,7</sup> Even with these systems, the best estimates suggest that the sensitivity approximates 60%.

Identification of *Candida* to the species level is essential, is more readily performed than routine susceptibility testing, and can be used to predict susceptibility patterns. A single positive blood culture is considered to be indicative of invasive disease and should not be considered a contaminant.<sup>8</sup> Culture from non-sterile sites is difficult to interpret. For example, urine culture positive for *Candida* may indicate either colonization or invasive disease in a febrile ICU patient.<sup>9</sup> The detection of  $\beta$ 1,3-D-glucan (Fungitell®) in plasma is FDA approved to detect fungal infection.<sup>8</sup>  $\beta$ 1,3-D-glucan is a structural component of the fungal cell wall and is not found in bacteria, viruses or mammals and its presence in the circulation can be an indicator of invasive fungal disease. It is a non-specific assay, becoming positive in a number of other invasive fungal infections, but the test can be reliably completed in approximately 2 hours. False positives can be caused by using cellulose membranes for hemodialysis, and transfusion of plasma components that have filtered with cellulose membranes.<sup>9</sup> Other serodiagnostic assays, including polymerase chain reaction (PCR) and real-time PCR methods, are being developed but are commercially unavailable. Histopathologic examination of biopsy material can be highly useful for diagnosis of disseminated infection, but identity of the specific pathogen can be very difficult. Peptide nucleic acid fluorescent in situ hybridization (PNA-FISH) has been used to aid diagnosis in tissue or blood, using probes specific for fungal RNA. These probes have been successful in differentiating certain *Candida* spp.<sup>9</sup> and this can be important in predicting antifungal susceptibility.

The clinical diagnosis of invasive candidiasis depends on a high index of suspicion based on well-recognized risk factors. Prolonged ICU stay, Acute Physiology and Chronic Health Evaluation II score >20, renal failure, hemodialysis, broad-spectrum antibiotics, central venous catheterization, parenteral nutrition, immunosuppression, severe acute pancreatitis, colonization with *Candida*, and surgery are all known risk factors for *Candida* infection.<sup>1</sup> Colonization does not always indicate infection; however, the absence of colonization with *Candida* makes invasive infection less likely. It is difficult to correlate the density of colonization with the likelihood of invasive candidiasis, but the presence of *Candida* spp. from one or more sites must be taken into account in the total clinical picture as an added risk for invasive disease. In all cases of proven or suspected invasive candidiasis, a dilated eye exam should be performed to look for evidence of chorioretinitis or endophthalmitis.<sup>10</sup>

In the past 20 years there has been a trend towards non-*albicans* spp. as a cause of disseminated *Candida* infections.

**Table 29-1** Fungi that may present as a systemic infection

<b>Fungus</b>	<b>Usual patient and clinical setting</b>	<b>Principal manifestations other than fever and fungemia</b>
<i>Candida</i> spp.	Intensive care unit patients (nonneutropenic patients)	Endophthalmitis
	Neonates	Meningitis
	Severely neutropenic and transplant patients	Nodular cutaneous lesions, hepatosplenic involvement
<i>Histoplasma capsulatum</i>	Acute DH: infants, immunosuppressed adults (e.g., lymphoma)	Hepatosplenomegaly, marrow involvement (anemia, leukopenia, thrombocytopenia), interstitial pneumonia
	Acute DH in AIDS patients: AIDS patients	Maculopapular rash, interstitial pneumonia, marrow involvement (anemia, leukopenia, thrombocytopenia), sepsis, disseminated intravascular coagulation
	Subacute DH: healthy and immunosuppressed adults	Undifferentiated fever, hepatosplenomegaly, marrow involvement (anemia, leukopenia, thrombocytopenia), oral ulcerations, adrenal deficiency
	Chronic DH: apparently healthy adults	Oropharyngeal ulcers or nodules, weight loss; hepatosplenomegaly in 30%
<i>Blastomyces dermatitidis</i>	Healthy or severe immunosuppression (e.g., AIDS)	Persistent mild pneumonia, chronic ulcerative skin lesions
<i>Coccidioides immitis</i>	Healthy or severe immunosuppression (e.g., AIDS)	Variable: skin, bone, central nervous system, and lung involvement are prominent
<i>Cryptococcus neoformans</i>	Severe immunosuppression (e.g., AIDS)	Central nervous system involvement and molluscum contagiosum —like rash
<i>Sporothrix schenckii</i>	Severe immunosuppression (e.g., AIDS)	Nodular skin lesions plus multifocal bone and joint involvement
<i>Paracoccidioides brasiliensis</i>	Male patient residing in Central or South America	Destructive lesions of the oropharynx or nares, bone involvement, lung involvement
<i>Aspergillus</i> spp.	Severely neutropenic or transplant patients	Fungemia is rare; pulmonary and central nervous system involvement are most common
<i>Fusarium</i> spp.	Severely neutropenic or transplant patients	Paronychia and nodular skin lesions that often become necrotic
<i>Trichosporon asahii</i>	Severely neutropenic or transplant patients	Nodular skin lesions may be seen
<i>Malassezia</i> spp.	Associated with use of lipid-rich total parenteral nutrition solutions	Undifferentiated fever
<i>Blastoschizomyces capitatus</i>	Severely neutropenic or transplant patients	Hepatosplenic involvement may develop
<i>Penicillium marneffei</i>	Travel to Southeast Asia or China, even if years previously, AIDS	Disease is similar to acute disseminated histoplasmosis
Other agents*	Severely neutropenic or transplant patients	Fever and fungemia

\**Paecilomyces* spp., *Hansenula* spp., *Saccharomyces* spp., *Rhodotorula* spp. DH, disseminated histoplasmosis.



Many non-*albicans* *Candida* species are associated with specific risk groups, for example *C. glabrata* in older patients and patients with neoplasms; *C. tropicalis* in patients with hematopoietic malignancy and/or neutropenia patients; *C. parapsilosis* in neonates and patients with chronic indwelling lines; and *C. krusei* in stem cell transplant recipients and leukemia patients who have received fluconazole prophylaxis.<sup>8</sup>

Infants in the neonatal ICU are at risk for disseminated candidiasis. Risk factors in this population include low gestational age, low APGAR scores and congenital malformations. *Candida parapsilosis* is the predominant non-*albicans* isolate in this setting, accounting for more than 30% of isolates, compared to 10–15% in adults.<sup>11</sup> Disseminated candidiasis in neonates occurs primarily in two forms, both of which are related to peripartum colonization. Congenital cutaneous infection is presumed to be from an ascending infection of the uterus. It occurs hours after birth<sup>12,13</sup> and is typified by a macular papular rash which can evolve into pustules, vesicles or desquamation. Samples from skin lesions may reveal the organism by culture and direct microscopy. In cases of prematurity or prolonged rupture of the membranes, cultures of CSF, blood, and urine should be obtained as these groups are at higher risk of disseminated infection.<sup>14</sup> Rarely, a second form of infection presents as a sepsis-like syndrome a few weeks after birth.<sup>12,15-17</sup> It is diagnosed by demonstrating fungemia and can involve the CNS, lungs, and skin.<sup>18</sup> Duration of candidemia >5 days is linked to an increased likelihood of ophthalmologic, renal, and cardiac involvement.

In the neutropenic adult patient, disseminated candidiasis is suggested clinically by newly developing subcutaneous nodules in a patient with undifferentiated fever. Biopsy of a characteristic lesion will reveal yeast histopathologically. In patients with persistent fever and neutropenia despite 4–7 days of broad-spectrum antibacterial therapy, empiric antifungal therapy should be initiated for possible candidiasis or other fungal disease. Early empiric antifungal therapy is more likely to succeed in neutropenic patients, as advanced infection is associated with considerable morbidity and mortality.<sup>19,20</sup> Hepatosplenic candidiasis (chronic disseminated candidiasis) may be seen in this population after the recovery from neutropenia. It is manifested by fever, right upper quadrant abdominal pain, and elevated alkaline phosphatase. Characteristic, well-demarcated lesions can often be seen on CT or ultrasound throughout the liver and spleen.<sup>21</sup>

Other metastatic complications of *Candida* infection in adults include endophthalmitis, endocarditis, myocarditis, pericarditis, and vertebral osteomyelitis.<sup>10</sup> Candidemia is associated with an overall mortality of 40% in adults and 20% in neonates and children, although most experts agree that the attributable mortality in these populations is 15–25% and 10–15%, respectively.<sup>4,11</sup>

## ***Aspergillus* spp.**

Invasive aspergillosis (IA) can be caused by several *Aspergillus* species, most commonly *A. fumigatus*, *A. flavus*, *A. niger*, and *A. terreus*. Patients at risk for *Aspergillus* infection include those with prolonged neutropenia, severe immunosuppression after allogeneic bone marrow transplant or solid organ transplant, prolonged exposure to steroids, and chronic granulomatous disease.<sup>22</sup> Stem cell transplant recipients receiving

prophylaxis or treatment for graft-versus-host disease (GvHD) are at particularly high risk.<sup>23</sup> Patients with advanced HIV are at risk for pulmonary infection, but seldom have disseminated infection.<sup>24</sup> *Aspergillus* infection has also been observed in ICU patients, especially in those with extensive trauma, steroid-dependent chronic obstructive pulmonary disease (COPD) or hepatic cirrhosis.<sup>25</sup> The most common invasive infections involve the lungs, skin, and/or sinuses. Central nervous system (CNS) involvement is a common manifestation of disseminated disease, although any organ may be involved.

Diagnosis relies on clinical suspicion in a patient at risk who has clinical evidence of pulmonary parenchymal invasion, sinusitis, unexplained skin and/or subcutaneous lesions, or typical radiographic findings. Blood culture is rarely positive, except in cases of intravenous drug abuse leading to prosthetic valve endocarditis with *Aspergillus*.<sup>26</sup> Typical chest computed tomography (CT) findings include nodular lung disease, the “halo” sign or haziness surrounding a pulmonary nodule, and necrotizing lesions associated with the “air crescent” sign.<sup>22</sup> However, these signs may be absent, particularly in non-neutropenic patients.<sup>25</sup> Positive culture from a sterile site or histopathologic confirmation is required for proven disease. Non-invasive diagnostic tests include the Platelia *Aspergillus* galactomannan ELISA, and are an approved adjunct to the diagnosis of IA. The test demonstrates population-dependent sensitivity and specificity,<sup>27</sup> and may produce false-positive results in the presence of piperacillin-tazobactam, imipenem and other  $\beta$ -lactam antimicrobials, and may be falsely negative in the setting of antifungal use.<sup>22</sup> The  $\beta$ 1,3-D-glucan assay (Fungitell®) can also be used to detect IA, but  $\beta$ -glucan is non-specific as this is a constituent of the cell wall of *Candida*, *Fusarium*, *Acremonium*, *Aspergillus* spp., and *Pneumocystis jirovecii*.<sup>27</sup>

## **Zygomycosis**

The zygomycetes are a class of fungi known to cause cutaneous, locally invasive, and disseminated infection. This class includes *Rhizopus* spp., *Rhizomucor* spp., *Absidia* spp., *Apophysomyces* spp., *Cunninghamella* spp., and *Mucor* spp. Infections are rarely seen in normal hosts, occurring almost exclusively in hosts with well-defined risk factors such as poorly controlled diabetes, metabolic acidosis, steroid therapy, solid organ and hematopoietic stem cell transplant, penetrating trauma, burns, neutropenia, iron overload, and deferoxamine therapy.<sup>28</sup> Many centers have seen a rise in incidence of opportunistic infection in neutropenic patients over the past 10 years with increasing use of voriconazole prophylaxis and changing chemotherapeutic regimens.<sup>29</sup> Locally invasive disease most commonly involves the lungs and/or sinuses but may involve any organ, including the GI tract or skin.<sup>30</sup> Disseminated disease may develop from locally invasive disease that spreads hematogenously.<sup>28</sup> Cases have also been reported related to intravenous (IV) drug abuse<sup>31</sup> and peritoneal dialysis catheters.<sup>28</sup> After dissemination, any organ may be affected.<sup>28,31,32</sup>

A presumed diagnosis is based on direct visualization of organisms in tissue biopsy of affected organs. A positive culture from affected tissue confirms the diagnosis. Both Platelia *Aspergillus* ELISA galactomannan and  $\beta$ 1,3-D-glucan assays are negative among patients with zygomycosis.<sup>28</sup> Culture of

tissue, blood or pulmonary secretions has traditionally been low yield, but some evidence shows that different methods of specimen preparation lead to improved recovery of the organism from cultures.<sup>33</sup> Culture is the primary method to determine genus and species, which can lead to different treatment decisions for these resistant organisms. PCR, in situ hybridization, and other identification methods are being developed to increase the efficiency and accuracy of diagnoses.<sup>34</sup>

### **Fusarium spp.**

*Fusarium* spp., most often *F. solani* or *F. oxysporum* or rarely *F. verticilloides*, *F. moniliforme* or *F. proliferatum*,<sup>35</sup> can cause keratitis, endophthalmitis, cellulitis, invasive sinusitis, pneumonia, septic arthritis, thrombophlebitis, and disseminated infection.<sup>36</sup> Risk factors for dissemination from pulmonary, skin or catheter-related sources include prolonged, profound neutropenia, hematologic malignancy, hematopoietic stem cell transplant, burns, and T cell deficiency.<sup>36-40</sup> Preexisting *Fusarium* onychomycosis may be a risk factor and this syndrome should be strongly suspected in any neutropenic patient with fever and a paronychia. The presentation of disseminated disease can mimic aspergillosis, but has a greater propensity to have skin lesions and positive blood cultures.<sup>35</sup> Fusariosis produces a disseminated maculopapular or nodular rash, commonly with necrosis and ulceration of the lesions resembling lesions of ecthyma gangrenosum.<sup>41</sup> This rash is present in 60–90% of cases.<sup>35,36,42</sup> The diagnosis can be made by biopsy and culture of a lesion or blood culture. About 60% of patients with disseminated fusariosis have positive blood cultures.<sup>43</sup>

### **Trichosporon spp.**

*Trichosporon* species are an uncommon but important cause of deep and disseminated infection. *T. asahii* and *T. mucoides* are thought to cause most deep infections, formerly attributed to *T. beigelii*.<sup>42</sup> Risk factors for deep or disseminated infection include leukemia, multiple myeloma, neutropenia, solid organ transplant, IV drug use, chronic active hepatitis, glucocorticosteroids, prosthetic valve surgery, HIV, chronic peritoneal dialysis, and burns.<sup>42,44-47</sup>

Hematogenously disseminated infection often presents with fungemia and multiple organ infection similar to *Candida* disseminated infection. Acute disseminated infection has a sudden onset and rapid progression. It most often occurs in the setting of neutropenia, manifesting as fever that is unresponsive to antibiotics, characteristic bullous hemorrhagic cutaneous lesions, pulmonary infiltrates, hypotension, renal or ocular involvement. Chronic disseminated infection presents more insidiously, with weeks or months of fever after recovery from neutropenia. It is typified by liver and/or spleen abscesses visible on CT or magnetic resonance imaging (MRI), similar in appearance to hepatosplenic candidiasis. Disseminated trichosporonosis may involve virtually any organ.

Diagnosis is made by culture of blood, urine or involved tissue. Because *T. asahii* has a glucuronylxylomannan cell wall moiety similar to that found in *Cryptococcus neoformans*, serodiagnostic kits that detect cryptococcal polysaccharide antigen are sometimes positive among patients with invasive trichosporonosis.<sup>44,48</sup>

### **Scedosporium spp.**

Two members of the genus *Scedosporium*, *S. apiospermum* (teleomorph *Pseudallescheria boydii*) and *S. prolificans* (syn. *S. inflatum*), have been associated with aggressive deep-seated infections in normal and immunocompromised patients.<sup>49-51</sup> The usual infections caused by *S. apiospermum* include sinusitis, endophthalmitis, otitis, endocarditis, pneumonia, and osteomyelitis. Dissemination to the CNS can produce brain abscess, epidural abscess or chronic meningitis.<sup>51</sup> Invasive lung disease is seen in neutropenic patients.<sup>42</sup> Pneumonia and disseminated disease have also occurred in association with near drowning.<sup>35</sup> The frequent involvement of sinuses and lungs points to a respiratory route as the main portal of infection entry.

*S. prolificans* may colonize the skin or respiratory tract, and is reported more frequently in Spain and Australia. Both immunocompromised and competent hosts can develop disseminated disease, usually manifested by fever, fungemia, skin lesions, myalgias, endophthalmitis, septic arthritis, pulmonary infiltrates, and/or CNS involvement.<sup>42</sup> In profoundly neutropenic patients it has been associated with rapidly fatal pneumonia and dissemination infections.<sup>35</sup> The diagnosis of either form of scedosporiosis is based on the isolation of the fungus from clinical specimens.<sup>51,52</sup>

### **Malassezia furfur**

Known primarily as the cause of pityriasis versicolor, *M. furfur* is a lipophilic yeast that rarely causes a sepsis syndrome. Disseminated infection is seen most often in infants or neonates who are receiving intravenous hyperalimentation with lipid supplementation,<sup>53,54</sup> and can also occur in immunocompromised adults with or without concomitant intravenous lipid supplementation.<sup>53,55,56</sup> Symptoms include fever, sepsis, multiorgan dysfunction, apnea, respiratory distress, bradycardia, hepatosplenomegaly, and lethargy. Findings include thrombocytopenia and leukocytosis.<sup>44</sup> A few cases of possibly hematogenously disseminated pneumonia have also been described.<sup>53</sup>

Culture of the organism is confounded because of its significant requirement for large amounts of lipid in the culture medium. If this organism is suspected on clinical grounds, the microbiologist can enhance recovery by use of either standard media supplemented with lipid or the lysis centrifugation system. Another *Malassezia* spp., *M. pachydermatis*, has been associated with systemic infection in humans.<sup>57</sup> In contrast to *M. furfur*, this organism does not require fatty acid supplementation for growth. The predisposing factors, as well as symptoms of infection, appear to be similar to those of *M. furfur*.

### **Miscellaneous agents**

A variety of uncommon yeasts have been reported to cause febrile syndromes, usually in conjunction with fungemia. *Blasotrichomyces capitatus* may cause disseminated infection in severely immunocompromised patients.<sup>58,59</sup> In one series, 16 persons with probable or possible infection were described,<sup>58</sup> all of whom had leukemia. Although any organ may be involved, pneumonia and focal hepatosplenic lesions were especially common. Hepatosplenic involvement was clinically

similar to that seen with hepatosplenic candidiasis. Diagnosis may be made by blood culture or examination of tissue biopsy.<sup>44</sup> *Rhodotorula* spp. (most often, *R. rubra*) have been well documented as causes of fungemia, CNS infection, peritonitis or endocarditis, especially in association with an intravascular catheter.<sup>60</sup> Diagnosis may be made by culture from a sterile site, as *Rhodotorula* is a frequent colonizer.<sup>44</sup> *Hansenula anomala* has been associated with intravenous catheter-related fungemia in the immunocompromised host,<sup>61</sup> endocarditis in an intravenous drug user,<sup>62</sup> and fungemia and cerebral ventriculitis in premature infants.<sup>63</sup> *Saccharomyces cerevisiae* may cause fungemia in association with intravascular catheters and prosthetic valves.<sup>64-66</sup> Such infections have most often been reported in severely immunocompromised patients and may lead to widespread visceral dissemination (liver, spleen, heart, and kidney).<sup>64,67</sup>

Although many moulds can produce disseminated infections, they are less likely to present with fungemia than with localized symptoms related to obvious localized infection. *Scopulariopsis brevicaulis* has been reported as a cause of endocarditis, but the organism was isolated only from the valve and related embolic material, not from the blood.<sup>68</sup> It has also been implicated in cases of mycetoma, keratitis, pneumonia, brain abscess, nasal septum invasion, and infection of traumatic wounds.<sup>35</sup> *Paecilomyces* spp., especially *P. lilacinus* and *P. variotii*, are common culture contaminants. They have been implicated in cases of fungemia in association with intravascular devices and prosthetic heart valves.<sup>69,70</sup> Cases of endocarditis, peritonitis, pyelonephritis, endophthalmitis, keratitis, orbital granuloma, skin lesions, and disseminated disease have been reported. Diagnosis is made by culture of blood or involved tissue.<sup>35</sup>

## Endemic mycoses and cryptococcosis

### *Histoplasma capsulatum*

*Histoplasma capsulatum* is a thermally dimorphic fungus that is found in soil enriched with nitrogen from decaying bird or bat droppings or other decaying organic matter. Exposure is often related to activities which disturb soil or droppings such as landscaping, building demolition or spelunking. In endemic areas it is often difficult to determine whether infection represents primary or reactivation disease.<sup>71</sup> Transmission from infected donor organs has also been widely reported.<sup>72</sup> It is poorly understood why certain patients have acute or chronic forms of this disease, but it is theorized to be related to inoculum size and host response to the pathogen.<sup>71,73</sup> Three syndromes of disseminated infection are recognized (see Table 29-1) and are described below.<sup>74</sup>

Acute disseminated disease is seen in infants and in immunosuppressed adults<sup>74</sup> due to advanced HIV infection, hematologic malignancy, or transplantation. Increased risk of infection has been seen with use of tumor necrosis factor antagonists and glucocorticosteroids. Symptoms include fever, chills, malaise, anorexia, weight loss, and shortness of breath. Skin lesions or, less commonly, mucous membrane ulcerations may be seen. The hallmark of this form of infection is massive involvement of the reticuloendothelial system, resulting in hepatosplenomegaly, lymphadenopathy, and bone marrow

involvement with pancytopenia. Patients may have diffuse reticulonodular lung involvement. Common laboratory findings are non-specific and include elevated alkaline phosphatase, extraordinarily high lactate dehydrogenase (LDH) and sedimentation rate. Patients with HIV are particularly prone to fulminant disseminated histoplasmosis with overwhelming sepsis, coagulopathy, respiratory failure from adult respiratory distress syndrome (ARDS), liver failure, renal failure, and CNS involvement.<sup>75</sup> In all patients, adrenal insufficiency may result from adrenal involvement. Gastrointestinal involvement ranges from intermittent to severe diarrhea associated with malabsorption.

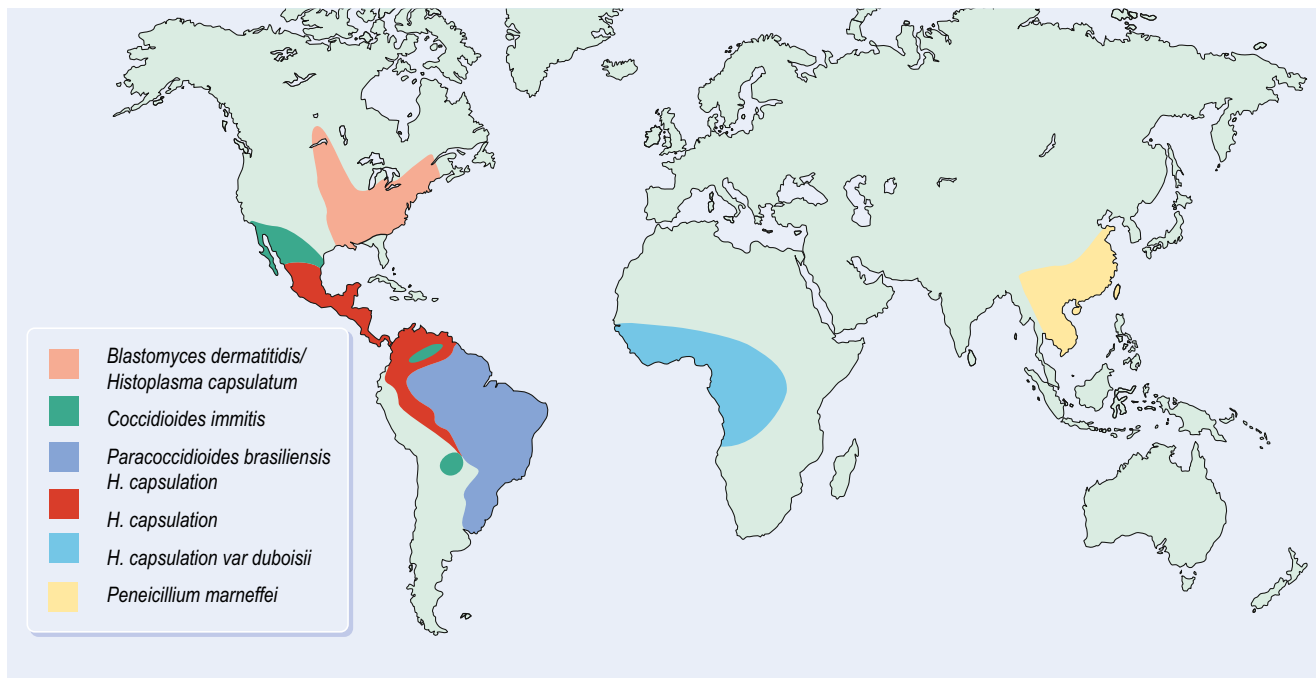
Subacute disseminated disease presents as a diagnostic dilemma. It occurs more commonly in older patients or those receiving chronic immunosuppressive therapies, but may occur in younger patients. Symptoms include fever and hepatosplenomegaly. Gastrointestinal involvement may lead to bleeding or oropharyngeal ulcers, and adrenal insufficiency may result from adrenal involvement. CNS involvement may present as chronic meningitis, mass lesion or cerebritis.<sup>74</sup> Other focal organ involvement may be seen such as endocarditis or other intravascular infection.

Chronic disseminated histoplasmosis is associated with very few symptoms and typically occurs in otherwise normal adults. Fifty percent have mild constitutional symptoms such as weight loss, malaise, lethargy, and intermittent fever. The most common symptom is a solitary, painful ulcer which can be anywhere in the upper respiratory or gastrointestinal tracts. The lesion may resemble a malignancy, but yeast can be visualized on histopathologic examination of biopsy specimens. Virtually any organ can be involved in chronic histoplasmosis, leading to chronic granulomatous hepatitis, chronic meningitis, osteomyelitis, adenitis, and endocarditis.<sup>72,73,76,77</sup>

Diagnosis of histoplasmosis can be made by histopathologic examination of tissue or culture from clinical specimens, especially blood or bone marrow. Cultures grown at physiologic temperatures may be reported initially as positive for yeast and later be identified as a dimorphic mould. In acute disseminated disease, examination of peripheral blood smear may identify organisms within leukocytes. Complement fixation looking for antibody to H and M antigens may be used.<sup>71</sup> H antibody positivity signifies active infection and M antibody positivity indicates past exposure.<sup>77-80</sup> Antibody detection is variable in immunocompromised patients. The ELISA for a cell wall polysaccharide antigen is more sensitive in disseminated disease, and can be measured in body fluids such as urine, blood, cerebrospinal fluid or bronchoalveolar lavage (BAL) fluid. The urine assay is the most sensitive, having a sensitivity of >90% in disseminated disease.<sup>73</sup> However, antigen is often not detected among patients with only pulmonary or mediastinal disease. This test may be false positive among patients with blastomycosis, paracoccidioidomycosis, and penicilliosis because of cross-reacting antigens present in the cell wall component of these organisms.<sup>81</sup>

African histoplasmosis is caused by *H. capsulatum* var. *duboisii*, which is endemic to a region of western and central sub-Saharan Africa (Fig. 29-1). It usually presents as focal bone or cutaneous disease with a wasting syndrome consisting of fever, weight loss, and pancytopenia.<sup>71,82,83</sup> Usual skin findings include ulcers, nodules or papulosquamous psoriatic-like lesions. Bone lesions are osteolytic and usually involve the





**Figure 29-1** Major geographic regional distribution of the endemic mycoses.

skull and/or ribs. Progressive, disseminated disease consisting of multiorgan involvement, fever, and hematologic abnormalities has been recognized. Disseminated disease with HIV co-infection has been reported and is characterized by fever, cutaneous lesions, and diffuse bone involvement.

### *Coccidioides* spp.

*Coccidioides* is a dimorphic fungus endemic to the southwestern US, northern Mexico, and focal areas in Central and South America (see Fig. 29-1). Disease is caused by two species: *C. immitis*, the organism responsible for coccidioidomycosis primarily in California, and *C. posadasii*, the organism responsible for disease primarily outside California. Epidemics in healthy hosts have been associated with specific climate conditions of moist soil followed by drought, then soil exposure by dust storm, earthquake or archeologic digging.<sup>84</sup> Infection is caused by inhaled arthroconidia and in normal hosts is frequently mild and self-limited. “Valley fever” is the typical infection, consisting of fever, cough, shortness of breath, pleuritic chest pain, fatigue, weight loss, and headache. It may be accompanied by a transient, fine, papular rash or, less commonly, by erythema nodosum or erythema multiforme. “Desert rheumatism” is erythema nodosum, arthralgias and fever and is not associated with disseminated infection. Typical incubation period is 1–3 weeks and recovery may take weeks to months, rarely with chronic complications.<sup>85</sup> Chronic complications include pulmonary nodules, cavities, ruptured cavities, mycetoma, and chronic fibrocavitary pneumonia.

Patients at risk for disseminated infection include immunosuppressed patients,<sup>86-90</sup> including transplant recipients, patients on chronic steroids, patients on TNF- $\alpha$  inhibitors, those with advanced HIV disease,<sup>85</sup> pregnant women, and patients of Filipino or African descent. Disseminated disease

may present as fulminant sepsis with multisystem involvement.<sup>84</sup> Chest x-ray may be normal or may show a diffuse reticulonodular pattern, especially in AIDS patients. The most common sites of disseminated infection include local invasion of pleura or pericardium, or hematogenous dissemination to the skin, joints, and meninges. Infections involving liver, spleen, peritoneum, prostate, and epididymis have been reported. Typical skin lesions are papules, nodules, abscesses, verrucous plaques or ulcers. Bone lesions are asymmetric and lytic, with predilection for weight-bearing joints and vertebrae, sometimes causing vertebral collapse and instability.

Diagnosis in non-endemic areas depends on travel history or potential exposure in the recent past or, in the case of newly acquired immunodeficiency, the remote past. Identification of organisms may be made by direct examination of biopsy or fluid with visualization of spherules. Cultures typically grow in 5–7 days, but must be handled under specific laboratory conditions as the mould form that grows in culture is contagious to laboratory workers. Serologic tests for tube precipitin antibodies and complement fixing antibodies are available, and are often useful in immunocompromised patients.<sup>88-90</sup> A positive antibody titer indicates infection, but HIV patients in particular may not have detectable antibody response, despite disseminated disease or meningitis.<sup>74</sup> Biopsy and culture of involved sites should always be pursued.

### *Blastomyces dermatitidis*

*Blastomyces dermatitidis* is a dimorphic fungus that lives in soil and decaying wood in south central and north central United States and Canada, parts of southern Europe and Africa (see Fig. 29-1). Infection is caused by inhalation of infectious conidia and rarely by inoculation.<sup>91</sup> Symptoms range from asymptomatic infection to severe multiorgan system

involvement and ARDS. Pulmonary infection is the most clinically recognized manifestation and can appear as acute or chronic pneumonia. Acute pneumonia can mimic bacterial or viral pneumonia, and chronic pneumonia can present with non-specific symptoms of fever, chills, weight loss, and cough productive of purulent sputum. The most common extrapulmonary site is the skin, presenting as lesions that are verrucous, papular or ulcerative. Blastomycosis can also present as osteomyelitis, prostatitis, epididymo-orchitis, and meningitis.<sup>91,92</sup> Immunocompromised patients may have an aggressive multiorgan system disease with high mortality.<sup>93</sup> These patients almost always have pulmonary findings that may consist of diffuse interstitial or alveolar changes or ARDS and respiratory failure. Verrucous or ulcerative skin lesions can be found in these patients.

Blastomycosis is considered a complication of advanced AIDS, usually occurring in patients with CD4 counts of less than 200.<sup>93</sup> It has also been observed in patients on cytotoxic chemotherapy and corticosteroid therapy, and in transplant recipients. Blastomycosis is infrequent in pregnant women, but perinatal transmission to the neonate with resulting severe infant infection has been reported.<sup>93</sup> Disease is much less common in children, but the manifestations of infection are similar to those seen in immunocompetent adults.

Diagnosis of blastomycosis may be difficult as the disease may mimic lung cancer, skin cancer, tuberculosis, histoplasmosis, nocardiosis, sarcoidosis and other granulomatous disorders. On chest roentgenogram, the infection may manifest as a nodule, cavitary lesion, mass lesion, lobar consolidation, atypical pneumonia, diffuse interstitial or miliary disease. Organisms may be directly visualized on examination of tissue, sputum, bronchial washings, pleural fluid, pus or urine sediment. *B. dermatitidis* is distinguished by its large, thick-walled, broad-based budding yeast forms. Culture may take up to 4 weeks for growth sufficient for identification, which may be confirmed using a DNA probe.<sup>94,95</sup> An EIA for *B. dermatitidis* A antigen, a complement fixation assay, and an immunodiffusion assay are available, but all lack sensitivity and specificity. A new *Blastomyces* antigen detection assay is most sensitive in urine, but can be performed on serum and cerebrospinal fluid as well. The assay is non-specific, cross-reacting with *Histoplasma*, *Paracoccidioides*, and *Penicillium* antigens.<sup>73</sup>

### *Sporothrix schenckii*

Infection due to *S. schenckii* most often causes disease limited to the skin after direct inoculation. Skin disease may spread proximally along lymphatics, exhibited by a line of nodular, ulcerative or plaque-like lesions.<sup>96</sup> Extracutaneous disease is also seen but is usually limited to a single site, with osteoarticular and pulmonary involvement being the most common sites.<sup>73,97,98</sup> Disseminated disease has been associated with alcoholism, diabetes, and COPD.<sup>98</sup> Osteoarticular disease often affects a single joint with predilection for the ankles, elbows, knees, and wrists. Infected joints appear swollen and painful, with effusions and possibly sinus tracts.<sup>97</sup> Pulmonary involvement has been associated with alcoholism, pulmonary tuberculosis, diabetes, sarcoidosis, and glucocorticosteroid use. It occurs after inhalation of conidia and causes fever, night sweats, weight loss, dyspnea, and cough productive of

purulent sputum. Chest roentgenogram may show unilateral or bilateral cavitary lesions, infiltrates, and hilar lymphadenopathy.<sup>98</sup> Chronic meningitis has also been described.<sup>96,99-102</sup>

A much smaller number of patients will present with multifocal extracutaneous sporotrichosis.<sup>96,97</sup> Such patients are almost uniformly immunosuppressed, typically with AIDS or a hematologic malignancy. The clinical picture is that of low-grade fever, weight loss, and mild anemia. Scattered skin lesions (usually nodular) may be present and dissemination to bone, joint, and the CNS is common. Infection may also cause a diffuse arthritis or tenosynovitis which may mimic disseminated gonococcal infection or seronegative spondyloarthropathy. Disseminated infection may also involve the palate, eye, liver, spleen, esophagus, colon, testes, bone marrow, or sinuses.<sup>97,101,102</sup> The infection is slowly but steadily progressive and frequently fatal.

The diagnosis can be established by culture of skin lesions, involved joints, blood, or bone marrow. In pulmonary disease examination or culture of sputum or bronchial washings may reveal organisms, but multiple specimens may be required. With arthritis or visceral involvement, tissue culture is often a better source for culture than synovial or other fluid.<sup>97</sup> In meningitis, large-volume CSF cultures may increase yield.<sup>96</sup> Histopathology reveals a mixed pyogenic, granulomatous reaction. Careful examination may reveal the characteristic cigar-shaped yeast, but organisms are sparse in most cases. In patients with advanced HIV disease the fungal burden may be significantly higher, making tissue biopsy or smears more useful. Documentation of the extent of systemic involvement can be obtained by use of nuclear bone and gallium imaging studies.<sup>103</sup> There are currently no useful serologic tests available for the diagnosis of sporotrichosis.

### *Paracoccidioides brasiliensis*

*Paracoccidioides brasiliensis* is a dimorphic fungus endemic to Central and South America (see Fig. 29-1). The primary site of infection is the lungs, but disease can lay dormant and reactivate up to 30 or more years after leaving an endemic area.<sup>104</sup> The incidence of disease is higher in men, with a mean ratio of 15:1. However, skin test positivity and disease in prepubertal children are equal among the sexes.<sup>104-108</sup>

Infection can be subclinical, resulting in conversion of the skin test. Disease is almost always disseminated at diagnosis<sup>104</sup> and comes in two major forms. The acute or subacute form is more common in children, adolescents, young adults and people with advanced HIV. It involves the reticuloendothelial system predominantly and is manifested by lymphadenopathy and hepatosplenomegaly with minimal, if any, pulmonary symptoms. The chronic form of disease more often affects adults and primarily involves the lungs. It presents with weight loss, fever, dyspnea, cough, mucosal ulcerations, and adrenal involvement. Chest x-ray is often disproportionately severe in comparison to symptoms, and typically demonstrates bilateral infiltrates sparing the apices. CT scan may reveal small cavities and fibrosis. Sequelae may include impaired pulmonary function and cor pulmonale. Both forms can involve skin with hypertrophic, ulcerative, nodular or acneiform lesions. Mucosal lesions are progressively destructive and painful and may involve the mouth, oropharynx, larynx, and rarely the anal or nasal mucosa. The adrenals may also become involved, rarely

causing Addisonian crisis. Other possible involvement includes chronic meningitis and osteolytic bone infection.<sup>107</sup>

Diagnosis may be made by visualization of the characteristic thick-walled yeast, demonstrating multiple buds in a “pilot wheel” configuration, from sputum, biopsy or other clinical sample.<sup>107</sup> Culture may require multiple samples to recover the organism and is slow growing. Antigen and antibody tests are available and may be useful in diagnosis of disease occurring in patients with relatively intact immune systems.<sup>104</sup> DNA probes have been used to more rapidly identify the organism from culture.

### *Penicillium marneffei*

*Penicillium marneffei* is a dimorphic fungus endemic to Southeast Asia and southern China (see Fig. 29-1). Although the precise reservoir is not known, the infection has been linked to exposure to bamboo rats. It may have a prolonged latency period, with an interval between exposure and diagnosis of up to 10 years.<sup>42</sup> *P. marneffei* can produce a disseminated infection in both healthy and immunocompromised hosts, but HIV coinfection has increased the prevalence of disseminated disease in endemic areas. It is the third most common opportunistic infection in HIV-infected patients in northern Thailand.<sup>109-111</sup>

The infection presents similarly to the syndrome of acute disseminated histoplasmosis.<sup>112</sup> Common symptoms include fever, weight loss, malaise, and skin lesions. As with acute disseminated histoplasmosis, involvement of the reticuloendothelial system and consequent anemia, leukopenia, lymphadenopathy, and hepatosplenomegaly are common. However, the frequent presence of multiple skin pustules, sometimes related to underlying necrotizing lymphadenitis, separates this infection from disseminated histoplasmosis. The skin lesions may have central umbilication that suggests molluscum contagiosum. Other manifestations may include mucosal lesions, diarrhea, colonic lesions, hemoptysis, osteoarticular disease, pericarditis, and pulmonary infiltrates.<sup>42</sup>

Suspicion of infection should be based on history of travel to or living in an endemic area. Diagnosis can be made by biopsy and culture of blood or any involved organ. The characteristic appearance of this organism in tissue is a small (2–4 µm) yeast with a distinct transverse septum easily identified in histopathologic specimens of blood or tissue. Other diagnostic tests remain experimental.

### *Cryptococcus* spp.

*Cryptococcus neoformans* and *C. gattii* are the most common pathogens in this genus.<sup>113,114</sup> In immunocompetent patients infection is usually limited to pneumonia, occasionally requiring antifungal treatment or, in persistent cases, surgical resection. Central nervous system involvement occurs occasionally in phenotypically normal patients. However, in immunocompromised patients, infection can involve pulmonary, CNS or other sites more frequently. Patients predisposed to severe and/or disseminated disease include those with advanced HIV, organ transplant recipients, patients with hematologic malignancy, chronic steroid recipients including patients with connective tissue, rheumatologic or chronic pulmonary disease, lung cancer, renal failure, hepatic cirrhosis, pregnancy or diabetes.<sup>113</sup> CNS involvement usually produces

clinically apparent meningitis, which may be acute, subacute or chronic. Acute pulmonary cryptococcal infection in immunocompromised patients may present as an asymptomatic nodule or lobar pneumonia, or as life-threatening pneumonia with ARDS. Chest x-ray may have nodules, cavities, infiltrates or consolidation. Less commonly, the x-ray will show pleural effusion, lymphadenopathy or endobronchial lesions. HIV patients may have disseminated disease with pneumonia and meningitis simultaneously,<sup>115</sup> and they may also have coinfection with other organisms such as atypical mycobacterium, cytomegalovirus, *Nocardia* or *Pneumocystis*.<sup>116</sup> Cutaneous involvement is also common among immunocompromised patients with disseminated cryptococcosis. The rash is polymorphic but a molluscum contagiosum-like, umbilicated, nodular rash is strongly suggestive of *C. neoformans*,<sup>117,118</sup> particularly among patients with AIDS. In other immunocompromised patients, cellulitis and myositis<sup>119,120</sup> are not uncommon presentations of disseminated disease.

Focal involvement of bone and joint,<sup>121-124</sup> eye,<sup>125</sup> kidney,<sup>126</sup> peritoneum<sup>127</sup> or prostate<sup>128</sup> may occur. Cryptococemia alone may be seen<sup>129-131</sup> among immunocompromised patients without obviously localizing signs. With recovery of immune function associated with reduction in immunosuppressive treatment, starting antiretroviral therapy or postpartum recovery, an immune reconstitution inflammatory syndrome (IRIS) may be observed with acute worsening of inflammation despite negative cultures.<sup>113</sup>

In addition to culture, diagnosis is by direct examination and/or culture of sputum, BAL fluid, CSF, or tissue for cytology and histopathology. Pathogenic *Cryptococcus* is distinctive due to the polysaccharide capsule, which is easily demonstrated on periodic acid-Schiff and mucicarmine stains. Following effective therapy, non-viable organisms may persist and still be visualized, but are not necessarily an indication of treatment failure. Assay for cryptococcal antigen can be performed on CSF and serum, and these antigen titers roughly correlate with burden of disease but are poor prognostic tools in general. In a patient with suspected cryptococcal meningitis, it is important to obtain opening pressure when lumbar puncture is performed, as managing increased intracranial pressure is of primary importance in decreasing morbidity and mortality from this infection.<sup>132</sup>

### Approach to the patient

When considered in the context of the patient and any secondary clues (or lack of secondary clues), the list of likely diagnoses can often be shortened to only one or two fungi (Table 29-2). Although it is doubtless true that most of the listed specific disease entities can be caused by any of the fungi, the entries in Tables 29-1 and 29-2 were selected because, even within the context of these relatively unusual diseases, these patterns of infection often suggest a specific diagnosis. None of the clues are pathognomonic, however, and the general principle in patients with a suspected disseminated fungal infection is to biopsy and culture all clinically involved areas. Blood cultures should be performed by the lysis centrifugation technique, as this appears to be more sensitive for fungi (especially *H. capsulatum*).<sup>7,133</sup> In addition, the laboratory should be asked to extend the incubation period of all cultures.<sup>134</sup> Serology



**Table 29-2** Diagnostic clues\*

Category/Clue	Organisms to Consider
<b>The Patient</b>	
Neonates	<i>Candida</i> spp., <i>Malassezia</i> spp.
Infants	<i>Candida</i> spp., <i>Histoplasma capsulatum</i> , <i>Malassezia</i> spp.
Nonneutropenic, critically ill adults	<i>Candida</i> spp.
Central venous catheterization	<i>Candida</i> spp.
Parenteral hyperalimentation	<i>Candida</i> spp., <i>Malassezia</i> spp.
Severe neutropenia, transplantation	<i>Candida</i> spp., <i>Aspergillus</i> spp., <i>Fusarium</i> spp.
AIDS, other cause of T-cell dysfunction	<i>H. capsulatum</i> , <i>Cryptococcus neoformans</i> , <i>Blastomyces dermatitidis</i> , <i>Coccidioides immitis</i> , <i>Penicillium marneffe</i>
<b>Skin Lesions</b>	
Papular or nodular	<i>Candida</i> spp., <i>H. capsulatum</i> , <i>Sporothrix schenckii</i> , <i>B. dermatitidis</i> , <i>Trichosporon beigelii</i> , <i>Fusarium</i> spp., <i>P. marneffe</i>
Ulcerative (cutaneous)	<i>B. dermatitidis</i> , <i>H. capsulatum</i> var. <i>duboisii</i>
Ulcerative (mucosal)	<i>H. capsulatum</i> , <i>H. capsulatum</i> var. <i>duboisii</i> , <i>Paracoccidioides brasiliensis</i>
Necrotic ulcer, suggesting ecthyma gangrenosum	<i>Aspergillus fumigatus</i> , <i>Fusarium</i> spp., agents of zygomycosis (e.g., <i>Rhizopus</i> spp.)
Nodular and umbilicated, suggestive of molluscum contagiosum	<i>C. neoformans</i> , <i>P. marneffe</i>
Pustules	<i>H. capsulatum</i> , <i>C. neoformans</i> , <i>C. immitis</i> , <i>S. schenckii</i> , <i>B. dermatitidis</i> , <i>P. marneffe</i>
Paronychia	<i>Fusarium</i> spp.
Subcutaneous nodules	<i>Fusarium</i> spp., <i>H. capsulatum</i>
Cellulitis	<i>Fusarium</i> spp., <i>C. neoformans</i> , <i>H. capsulatum</i>
<b>Travel</b>	
Southwest desert region of North America	<i>C. immitis</i>
North America's Midwest and Tennessee-Ohio river valley, Central America, South America	<i>H. capsulatum</i>
Eastern United States and Canada	<i>B. dermatitidis</i>
Latin America	<i>P. brasiliensis</i> , <i>H. capsulatum</i> , <i>C. immitis</i>
Southeast Asia or China	<i>P. marneffe</i>
Western and central sub-Saharan Africa	<i>H. capsulatum</i> var. <i>duboisii</i>
<b>Syndromes</b>	
Fever alone in nonneutropenic patient	<i>Candida</i> spp., <i>H. capsulatum</i> , <i>C. neoformans</i>
Fever alone in neutropenic patient	<i>Candida</i> spp., any opportunistic yeast or mould

(Continued)

**Table 29-2** Diagnostic clues\*—cont'd

Category/Clue	Organisms to Consider
Endocarditis	<i>Candida</i> spp., <i>H. capsulatum</i> , <i>Aspergillus</i> spp.
Reticulonodular pulmonary infiltrate and immunocompromise	<i>H. capsulatum</i> , <i>B. dermatitidis</i> , <i>C. immitis</i>
Adrenal insufficiency	<i>H. capsulatum</i> , <i>P. brasiliensis</i>
Oral ulceration, gastrointestinal bleeding	<i>H. capsulatum</i> , <i>P. brasiliensis</i> (oral ulcers only)
Bone or joint involvement	<i>C. immitis</i> , <i>S. schenckii</i> , <i>B. dermatitidis</i> , <i>P. brasiliensis</i> , <i>Scedosporium</i> spp., <i>H. capsulatum</i> var. <i>duboisii</i>
Hepatosplenomegaly in the nonneutropenic patient	<i>H. capsulatum</i> , <i>P. marneffeii</i> , <i>P. brasiliensis</i>
Hepatosplenomegaly (with or without kidney involvement) in the severely neutropenic patient	<i>Candida</i> spp., <i>Blastoschizomyces capitatus</i> , <i>T. asahii</i>
Peritonitis	<i>Candida</i> spp., <i>C. neoformans</i>
Meningitis	<i>C. immitis</i> , <i>C. neoformans</i> , <i>H. capsulatum</i>
Vascular thrombosis (stroke, myocardial infarction, Budd-Chiari syndrome)	<i>Aspergillus</i> spp., agents of zygomycosis (e.g., <i>Rhizopus</i> spp.)
Renal failure in neutropenic patients	<i>Candida</i> spp.
Endophthalmitis	<i>Candida</i> spp.

\*Although many other syndromes and physical findings have been described with each of the fungi, those listed in this table have been chosen because of their especially strong linkage with the indicated fungi.

should be sent when available, such as urine *Histoplasma* antigen for suspected histoplasmosis, serum cryptococcal antigen for cryptococcal infections, or serum  $\beta$ 1,3-D-glucan to screen for invasive fungal infection.

## Fever without any other manifestations

*Candida* spp. and *H. capsulatum* commonly present with fever alone. In invasive candidiasis the patient is often critically ill in an ICU, and the diagnosis is suspected by exclusion of other sources of fever in association with the usual risk factors. Disseminated candidiasis is also especially likely in neutropenic patients. In disseminated histoplasmosis, persistent fever in a patient with AIDS or another condition associated with severe cellular immune depression who is from an endemic area should lead one to consider the diagnosis. In the proper settings (see Table 29-2), infection due to *Trichosporon* spp. and *M. furfur* should also be considered in the febrile patient with no other clues regarding source. Finally, cryptococcosis can occasionally present with little more than fever and cryptococcoma.

## Cutaneous involvement

Many of the fungi can produce cutaneous manifestations as part of systemic involvement. This area has been the focus of several reviews.<sup>41,135-137</sup> Although some generalizations can be made about skin lesions that are associated with certain

fungi (see Table 29-2), it is safe to say that most of the fungi can produce a wide variety of skin manifestations. Thus, in the patient with a suspected systemic fungal infection, all skin abnormalities should be considered suspect, no matter how typical they are of other processes. This is especially true when the patient is severely immunosuppressed due to AIDS. Although chronic verrucous or ulcerative lesions may naturally bring fungi to mind, many other patterns have been described. For example, grouped vesicles that mimic herpes simplex infection have been reported with both cryptococcosis and histoplasmosis;<sup>135</sup> molluscum contagiosum-like lesions are now well described with cryptococcosis<sup>138</sup> and disseminated penicilliosis,<sup>112</sup> and acneiform rashes have been described with disseminated paracoccidioidomycosis.<sup>106</sup> Biopsy of suspicious lesions for culture and direct examination is required for proper diagnosis.

## Overwhelming infection and septic shock

In critically ill adults in the ICU, disseminated candidiasis may produce the picture of septic shock without localizing signs. Similarly, in patients with AIDS, disseminated histoplasmosis may produce an overwhelming and rapidly fatal septic picture.<sup>75</sup> Acute pulmonary coccidioidomycosis may produce a picture that strongly suggests bacterial pneumonia with septic shock.<sup>139</sup> Finally, disseminated aspergillosis and fusariosis may present as septic shock among patients with severe and prolonged neutropenia.

## Meningitis and other neurologic findings

*Cryptococcus neoformans* and *Coccidioides immitis* are the most common causes of fungal meningitis. Although the presentation may be striking, it is important to also appreciate that cryptococcal meningitis may present with little or no headache, without fever, and in association with slow onset of confusion or personality change.<sup>129</sup> Space-occupying cryptococcomas are also seen.<sup>140</sup> Meningitis due to *C. immitis* is usually clinically apparent and strongly suggested by the epidemiologic history.<sup>141</sup> CNS involvement due to *H. capsulatum* may vary from meningitis to focal neurologic deficits,<sup>142</sup> as may CNS involvement due to *B. dermatitidis*.<sup>92,143</sup> In neutropenic patients, infection due to *Aspergillus* spp., *Fusarium* spp., and other angioinvasive moulds can also produce space-occupying CNS lesions.

## Peritonitis

Although *Candida* spp. are relatively frequent causes of peritonitis in patients receiving peritoneal dialysis<sup>144</sup> and can disseminate from this site, this syndrome usually presents no diagnostic difficulties. Likewise, *C. neoformans* can also produce dialysis catheter-related peritonitis. On the other hand, *C. immitis*, *C. neoformans*, and *H. capsulatum* can all cause peritonitis in non-dialysis patients as part of a disseminated infection, and this may be especially true in patients with AIDS.<sup>145</sup>

## Renal failure

Renal failure may occur with any disseminated fungal infection but is most often seen among patients with disseminated candidiasis and may represent either direct invasion of the kidneys or ureteral obstruction. Most of these patients have candiduria, and renal failure may paradoxically improve when systemic antifungal therapy is given.

## Geography

Although many fungi have a worldwide distribution (e.g., *Candida* spp., *A. fumigatus*), others are only acquired in restricted geographic regions (see Fig. 29-1). The absence of travel in or near the endemic areas for fungi such as *C. immitis*, *P. brasiliensis*, *P. marneffeii*, and *B. dermatitidis* largely eliminates these fungi from consideration. *H. capsulatum* is present worldwide but is much more frequently seen in certain parts of the Americas.

## Conclusion

Hematogenously disseminated mycoses are increasingly important causes of morbidity and mortality, particularly among immunocompromised patients. The number of fungal pathogens that have the potential to cause disseminated disease has increased dramatically in the recent past. A high index of suspicion in the right clinical setting, appropriate diagnostic studies, and early aggressive antifungal therapy are necessary to offer patients the best chance of a successful outcome.

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# Fungal infections of the eye

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Ocular mycoses are recognized as an important worldwide cause of morbidity and blindness. Although fungal keratitis is the most common encountered clinical entity, other ocular structures, including the retina and orbital soft tissues, may also be affected. Ocular fungal infections without a systemic infectious component typically fall into the domain of the ophthalmologist and are not frequently encountered or managed by non-ophthalmologist clinicians. On the other hand, patients with sinoorbital mycoses are likely to present to clinicians who are not ophthalmologists. Since sinoorbital infection is a potentially life-threatening problem, prompt recognition of this disease by a family practitioner or an internist can lead to early intervention and a decrease in both morbidity and mortality.

## Fungal retinitis and endophthalmitis

### Anatomy

The anterior globe consists of the transparent cornea which inserts into the sclera at the limbus. The sclera is the white, collagenous outer layer of the eye, which is continuous with the cornea anteriorly and the dural sheath of the optic nerve posteriorly (Fig. 30-1).

The vascular, middle compartment of the eye, the uveal tract, consists of the iris, ciliary body, and choroid. The iris is the anterior extension of the ciliary body. It has a flat surface with a central round aperture, the pupil. The crystalline lens is suspended by fine zonules and rests within a capsular “bag”; the lens is positioned immediately posterior to the iris within the eye. The anterior chamber of the eye is the anatomic space between the anterior surface of the iris and the posterior surface of the cornea. The anterior chamber is filled with aqueous humor, a secretory product of the ciliary body. Situated in the iris stroma are the dilator and sphincter muscles of the iris that control the size of the pupil (Fig. 30-2).

The ciliary body extends from the anterior choroid to the root of the iris. It consists of a corrugated anterior portion, the pars plicata, and a flattened posterior portion, the pars plana. The choroid is the posterior portion of the uveal tract,

sandwiched between the retina and the sclera. The choroid consists of a rich vascular network known as the choriocapillaris.

The retina is a thin multilayered sheet of neural tissue that lines the inner wall of the posterior eye. The outer surface of the retina is apposed to the retinal pigment epithelium, while the inner surface is apposed to the vitreous. There are nine anatomically defined layers of neurosensory retina. The outer retinal layers receive blood from the choriocapillaris (i.e., the choroid), while the inner retina receives its blood supply from branches of the central retinal artery.

The vitreous is a transparent, avascular, gelatinous structure that occupies two-thirds of the volume and weight of the eye. It is intimately attached to the anterior peripheral retina and around the optic nerve by a fine scaffolding of collagenous fibers. The vitreous is 99% water, with the remaining 1% consisting of collagen and hyaluronic acid which give the vitreous its gel-like consistency.

Inflammation of the uveal tract is referred to as uveitis, and may involve one or all three portions of the uvea. In most intraocular infections the uveal tract is involved; however, the primary focus of infection is often one of the other ocular structures. Ocular infections are described by the anatomic structures involved. For example, a primary infectious process of the retina with secondary involvement of the choroid is descriptively termed retinochoroiditis.

The term “endophthalmitis” is reserved for describing a panophthalmic infectious or inflammatory process. Infectious endophthalmitis can be caused by either exogenous or endogenous microbial contamination of intraocular tissues. Exogenous endophthalmitis is usually associated with penetrating injury to the eye, although exogenous endophthalmitis can also result from contamination of the internal eye by surgical instruments, fluids, and foreign materials introduced into the eye during surgery.

In contrast, endogenous endophthalmitis is principally the result of hematologic spread of microorganisms from a distant focus of infection. Endogenous endophthalmitis occurs most often in immunocompromised patients, including intravenous drug abusers, patients receiving chemotherapy or total parenteral nutrition, as well as organ transplant recipients and HIV-positive patients.



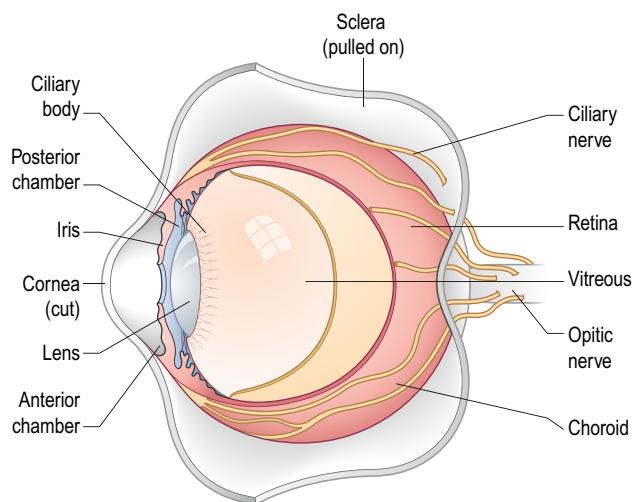


Figure 30-1 Anatomy of the eye.

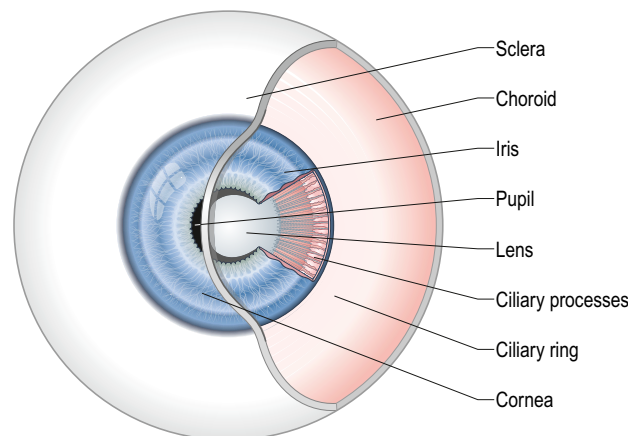


Figure 30-2 Anatomy of the eye

## Epidemiology

The incidence of all forms of exogenous endophthalmitis following penetrating trauma to the eye is approximately 5%, with more than 10% of these cases being of fungal etiology. In contrast, the incidence of exogenous endophthalmitis following intraocular surgery is extremely low, 0.05–0.2%,<sup>1,2,3</sup> with approximately 5% of these cases being due to fungus.<sup>4</sup> Obviously, the exposure of traumatized eyes to exogenous materials contaminated with bacteria and fungi accounts for the difference in incidence rates between trauma and surgery-induced exogenous endophthalmitis. Trauma involving organic foreign matter should always raise concerns about fungal contamination of the eye.

In contrast to exogenous endophthalmitis, more than 50% of endogenous endophthalmitis cases are caused by fungi.<sup>5,6</sup> In general, any patient group susceptible to opportunistic infections is at increased risk for endogenous fungal endophthalmitis. Defined risk factors for developing endogenous fungal endophthalmitis include  $\beta$ -D-glucan  $\geq 20$  pg, intravenous hyperalimentation, major surgery, fever of unknown origin refractory to antibiotic treatment, malignancy, and neutropenia ( $\leq 500/\text{ml}$ ).<sup>7</sup> For example, endogenous fungal endophthalmitis has been reported in up to 4% of patients receiving intravenous hyperalimentation.<sup>8</sup>

*Candida* spp. are responsible for the majority of endogenous endophthalmitis cases. *Candida* endophthalmitis has been reported in association with hyperalimentation, gastrointestinal surgery, corticosteroid therapy, and lymphomas. Diabetes has also been reported as a predisposing condition for development of *Candida* endophthalmitis.<sup>9</sup>

The prevalence of endogenous fungal endophthalmitis in patients with candidemia has been reported to be as high as 28–45%.<sup>10,11</sup> However, it is likely that these high prevalence rates reflect a broad definition of endophthalmitis in these studies and not necessarily true endophthalmitis. A prospective study of 118 patients with candidemia by Donahue<sup>12</sup> found no evidence of endophthalmitis in their study group. The following clinical criteria were used to define ocular infections in this study.

- Patients with intravitreal fluff balls or observable vitreal extension of chorioretinal infiltrates were classified as having endophthalmitis.
- Patients with chorioretinal lesions not associated with vitreous abscess or vitreous extension were classified as having *Candida* chorioretinitis; these lesions have been demonstrated histopathologically to contain *Candida*.
- Patients with intraretinal hemorrhages, nerve fiber layer infarcts, and/or white-centered hemorrhages (Roth spots) without chorioretinal infiltrates were classified as having non-specific lesions because such signs may have causes other than infection. For example, nerve fiber layer infarcts can be a manifestation of either poor ocular perfusion, as would be expected in a group of severely ill patients such as those with candidemia, or hypertension, diabetes mellitus, anemia, collagen vascular disease, or lymphoproliferative states.

Despite the absence of documented endophthalmitis in Donahue's study, *Candida* chorioretinitis was observed in 9.3% of patients; an additional 20% of patients had fundus lesions that were classified as non-specific. This 29% prevalence rate of "ocular findings" in candidemic patients is comparable to the "endophthalmitis" prevalence rate observed in earlier studies, with less strict inclusion criteria. Most likely, hematogenous spread of *Candida* to the choroid and/or retina in candidemic patients is common but endophthalmitis is quite rare. Undoubtedly, more efficacious antifungal therapy and prompt treatment play a role in the progression from chorioretinitis to endophthalmitis.

*Aspergillus* endophthalmitis is most common in organ transplant recipients, neutropenic patients, patients receiving chemotherapeutic agents or corticosteroids, and those undergoing valvular cardiac surgery.<sup>9,13</sup> One report of autopsy results in liver transplant recipients demonstrated evidence of *Aspergillus* endophthalmitis in seven cases; interestingly, only one of these seven cases had clinically recognized endophthalmitis.<sup>14</sup> One nosocomial outbreak of *Aspergillus* endophthalmitis was linked to hospital construction.<sup>15</sup>

## Pathogenesis

### Endogenous fungal endophthalmitis

Endogenous endophthalmitis likely starts by hematogenous spread of the fungus to the choroid followed by contiguous spread to the adjacent retina. The high blood flow to the choroid and outer retinal layers (150 mm/s), compared to a lower flow to the inner retinal layers (25 mm/s), makes this tissue vulnerable to both infectious as well as metastatic seeding.

Histologically, there is an acute suppurative inflammation composed mainly of neutrophils. Tissue samples have shown abundant levels of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase in intraocular structures with fungal endophthalmitis.<sup>9</sup> It has also been demonstrated that retinal pigment epithelium (RPE) generates a protein, retinal pigment epithelium protective protein (RPP), in order to suppress superoxide generation by neutrophils.<sup>9</sup> Since the elimination of *Aspergillus* requires functioning neutrophils, this microbe is found in subretinal and sub-RPE tissues where the combination of antioxidant enzymes and RPP allows the growth of fungi despite the infiltration of neutrophils. On the other hand, *Candida* requires nutrients such as glucose and low pH and therefore readily thrives in the vitreous where these conditions are present.<sup>9</sup>

### Exogenous fungal endophthalmitis

Cataract removal with intraocular lens implant and corneal transplantation are the ocular surgical procedures most often associated with postoperative fungal endophthalmitis. Contaminated irrigating solutions, intraocular lenses, ventilation system, and hospital construction activity have been reported as potential underlying causes of postsurgical exogenous fungal endophthalmitis.<sup>15-17</sup> *Candida* is the most common cause of postsurgical exogenous fungal endophthalmitis.<sup>18</sup> *Fusarium* is the most commonly identified etiologic agent of traumatic exogenous endophthalmitis.<sup>19</sup>

## Mycology

### *Candida* species

*Candida albicans*<sup>20</sup> is the most frequently isolated species although other *non-albicans* species have been implicated, including *C. parapsilosis*,<sup>17,21,22</sup> *C. krusei*,<sup>23,24</sup> and *C. tropicalis*.<sup>25</sup>

### *Aspergillus* species

*Aspergillus* spp. are the second most common fungal genus responsible for endogenous endophthalmitis,<sup>26</sup> with *A. flavus* the most frequently encountered species.<sup>27</sup> Other *Aspergillus* species include *A. fumigatus*, *A. niger*, *A. terreus*, *A. glaucus*<sup>28</sup> and *A. nidulans*.<sup>29</sup> *Aspergillus* demonstrates tropism for vascular tissue with angioinvasion of the hyphae observed in pathologic specimens.<sup>13</sup>

Table 30-1 reviews other fungi causing endophthalmitis.

## Clinical manifestations

The most common ocular symptoms of fungal endophthalmitis are redness, pain, and diminished or blurred vision in the involved eye. Examination of the external eye typically shows hyperemia of the ocular surface with dilation of the surface vessels. Occasionally, hypopyon may be visible which is a

**Table 30-1** Fungi causing endophthalmitis

Fungus	Patient Characteristics
<i>Candida</i> spp.	Diabetes mellitus, neutropenia, hyperalimentation, gastrointestinal surgery, prior antibacterial agents
<i>Aspergillus</i> spp.	Transplant recipients, neutropenia
<i>Fusarium</i>	Neutropenia, intravenous drug abuse, AIDS <sup>30-32</sup>
<i>Cryptococcus neoformans</i>	AIDS with disseminated <i>Cryptococcus</i> <sup>33</sup>
<i>Penicillium</i>	Intravenous drug abuse, <i>Penicillium</i> -related endocarditis <sup>34</sup>
<i>Coccidioides immitis</i>	Patients with disseminated disease but may occur in otherwise healthy individuals <sup>35</sup>
<i>Blastomyces dermatitidis</i>	Infrequent cause of human fungal endophthalmitis <sup>36</sup> Frequent cause of canine endophthalmitis

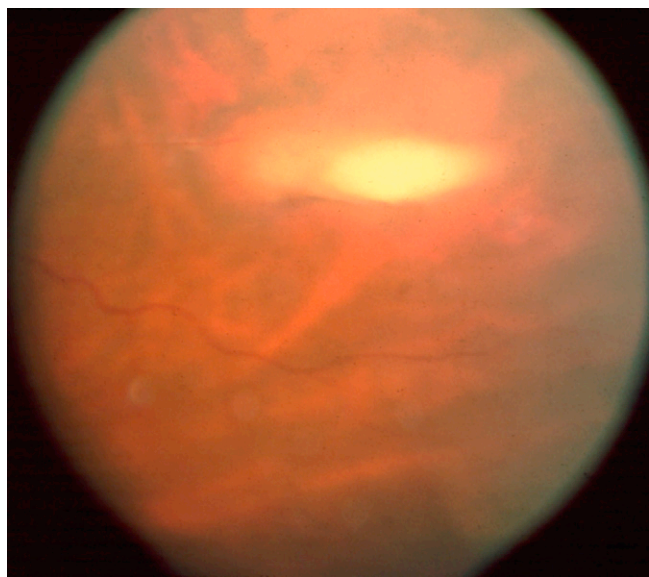
whitish layer of inflammatory cells and debris in the inferior anterior chamber.

Biomicroscopic examination of the eye with the slit lamp may reveal white blood cells and flare in the anterior chamber, as well as vitreous haze. Flare refers to protein leaking into the normally optically clear aqueous from incompetent intraocular vessels. Flare and white blood cells in the aqueous are indicative of ocular inflammation. Similarly, vitreous haze indicates loss of clarity due to protein and cellular debris in the usually optically clear vitreous. The presence of haze and white blood cells in the vitreous is indicative of inflammation or infection in the choroid, retina or both, with extension of the process into the vitreous.

Fundus examination by indirect ophthalmoscopy reveals chorioretinal infiltrates. These infiltrates appear as pale or creamy lesions in the ocular fundus (Fig. 30-3). The infiltrates obscure the normal underlying vascular flush of the choroid and details of the overlying surface retinal blood vessels. They are highlighted by the surrounding uninvolved retina with its normal vascular flush and architecture (see Fig. 30-3).

Endogenous fungal endophthalmitis develops in four clinical stages: (1) chorioretinal changes without extension into the vitreous cavity, i.e., chorioretinitis; (2) chorioretinal fungal mass penetrating through the inner limiting membrane of the retina into the vitreous cavity; (3) vitreous opacity (extensive vitreous infection) which blurs the view of the fundus; and (4) stage 3 with associated retinal detachment.<sup>7</sup>

A retinal detachment would be diagnosed by ultrasonography when vitreous opacity precludes direct examination. It has been reported that the stage of endophthalmitis noted at the time of initial examination correlates with prognosis, and treatment at an early stage results in better visual outcome.<sup>37</sup>



**Figure 30-3** *Candida* chorioretinitis. Note the creamy white appearance of the retinal infiltrate. Loss of retinal vasculature indicative of retinitis. No vitritis is present in this case.

Thus in patients susceptible to opportunistic infections, any new visual symptoms should prompt immediate consultation with an ophthalmologist to allow for early recognition.

## Diagnosis

The definitive diagnosis of infectious endophthalmitis is established by culture of the aqueous and/or vitreous fluids. Aqueous fluids can be obtained at the bedside or in the examining room using the slit lamp. With the use of topical anesthetic, a 27 or 30 gauge needle can be passed through the peripheral cornea at the limbus into the anterior chamber to withdraw 0.1 ml of aqueous fluid.

Vitreous fluid sample can also be obtained in the examining room or in the operating room using a 27-gauge needle introduced at the pars plana to withdraw ~0.2 ml of vitreous. The pars plana is the anatomic region of the choroid without overlying retina; it is situated 3.5 mm posterior to the limbus in pseudophakic or aphakic eyes and 4.5 mm posterior to the limbus in phakic eyes. A needle passed through the pars plana should not perforate the retina, thereby avoiding the risk of retinal puncture and possible retinal detachment. A vitreous sample is routinely obtained during pars plana vitrectomy when surgical intervention for endophthalmitis is indicated. Vitrectomy has the advantage of producing a larger sample volume, debulking the vitreous of toxic inflammatory and microbial products, and releasing traction on the retina.

Aqueous and vitreous samples should be plated on blood agar, chocolate agar, Sabouraud dextrose agar, thioglycollate broth, and anaerobic medium. In addition, Gram and Giemsa stains should be performed routinely on clinical samples. The remainder of the specimen, if any, should be mixed with an equal volume of 95% alcohol and submitted for pathologic study. When available, the polymerase chain reaction can provide rapid identification of the infecting organism in aqueous or vitreous samples.

## Treatment

All forms of endophthalmitis can lead to complete loss of vision of the infected eye, even with appropriate treatment. Since destruction of the delicate ocular tissues, particularly the retina, can occur rapidly, the importance of early recognition and intervention cannot be overemphasized. Retinal surgeons are uniquely qualified to perform interventional surgery such as vitrectomy. This procedure allows for acquisition of specimens, concomitant intravitreal administration of antimicrobials and reduction of the microbial load and toxic byproducts of infection and inflammation. Prompt diagnosis and treatment improve the chance of visual recovery, therefore aggressive diagnostic and therapeutic management is pursued in all suspected cases.

### Treatment for *Candida*

The successful use of parenteral amphotericin B was first reported for a case of *Candida* endophthalmitis in 1960,<sup>38</sup> although its efficacy was later determined to be limited because of poor penetration into the vitreous.<sup>17</sup> O'Day and associates found that the intravitreal concentration of amphotericin B after intravenous administration barely reached the minimum inhibitory concentration (MIC) against *Candida*.<sup>39</sup> However, intravitreal administration of amphotericin B (0.005 mg/0.1 ml), with or without pars plana vitrectomy, has been successfully employed for *Candida* endophthalmitis.<sup>40,41</sup> Intravitreal amphotericin B must be used with caution as even low concentrations (4.1 µg/ml or 8.3 µg/ml) have been reported to cause focal retina necrosis.<sup>42</sup>

The superiority of fluconazole to amphotericin B for *Candida* endophthalmitis has never been assessed by randomized study but due to the toxicity of amphotericin B, fluconazole has become widely used for *Candida* endophthalmitis.<sup>43</sup> Fluconazole can be given both systemically, with good intraocular penetration, as well as intravitreally. The main drawback of fluconazole is its lack of activity against some non-*albicans* *Candida* species.

Voriconazole, a second-generation synthetic derivative of fluconazole, has been employed for intravitreal treatment of fungal endophthalmitis. The depletion of ergosterol by the action of voriconazole leads to disruption of fungal cell membrane and cell lysis.<sup>44,45</sup> Voriconazole is more active than amphotericin B, fluconazole, itraconazole, and flucytosine against all *Candida* species.<sup>46</sup> *Candida albicans* is highly susceptible to this agent with a MIC<sub>90</sub> of only 0.06 µg/ml, while *C. glabrata* is the least sensitive, with a MIC<sub>90</sub> of 2.0 µg/ml.<sup>47</sup> Intravitreal voriconazole has been demonstrated to be effective in cases of amphotericin B- and fluconazole-resistant fungal endophthalmitis.<sup>48</sup> Azole-resistant *C. albicans* has been reported in endophthalmitis.<sup>49</sup> Promising results with combined intravenous voriconazole and intravenous caspofungin have been reported.<sup>50,51</sup> On the other hand, failure with caspofungin has also been reported in which inadequate vitreous concentrations were found.<sup>52</sup>

Some experimental evidence suggests a benefit to using intravitreal corticosteroids simultaneously with antifungal agents.<sup>53</sup> Although corticosteroids can minimize ocular inflammation, they may also predispose to progression of infection. Thus, until comparative studies demonstrate benefit, the use of intravitreal steroids cannot be recommended.



### Treatment for *aspergillus*

The prognosis for *Aspergillus* endophthalmitis is poor.<sup>54</sup> The use of systemic amphotericin B has been inconsistent because of its penetration into the eye. Vitrectomy with concomitant intravitreal amphotericin B has been used but with inconsistent success. Oral flucytosine and oral fluconazole as an adjunct to intraocular therapy have been successful in anecdotal reports.<sup>54</sup> Intravenous voriconazole and caspofungin with or without intravitreal voriconazole may become the standard for treatment of *Aspergillus* endophthalmitis.<sup>55</sup>

## Fungal keratitis

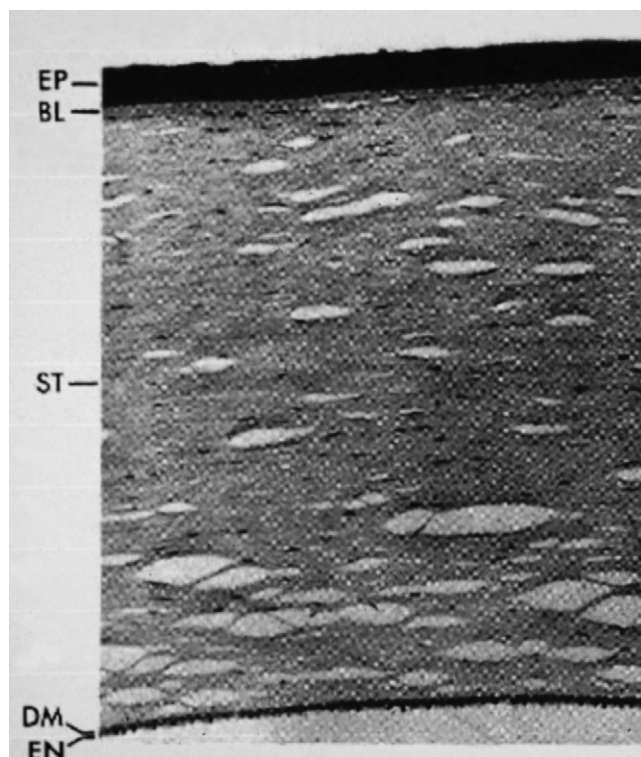
### Anatomy

The cornea is the transparent, avascular anterior-most structure of the eye, measuring 10–12 mm in diameter and having a central thickness of about 0.5 mm. It functions as an anterior refractive surface which contributes nearly three-quarters of the total refractive power of a normal human eye. Like the skin, it is the external anatomic barrier between the environment and deeper tissues; it is the ocular structure most frequently damaged by external trauma.

Anatomically, the cornea is divided into five layers (Fig. 30-4):

- the epithelium is composed of non-keratinized, stratified squamous cells and accounts for 5% of total corneal thickness. Tight junctions between epithelial cells prevent the penetration of tear film into the corneal stroma and also significantly limit drug penetration into deeper tissues
- Bowman's layer is a thin avascular layer underlying the epithelium
- the stroma, an extracellular matrix composed of proteoglycans, principally dermatan sulfate and keratan sulfate, collagen fibrils (types I, V, and VI), fibroblasts (keratocytes), and mucoproteins. The stroma accounts for 90% of the corneal thickness
- Descemet's membrane, which is the basement membrane of the corneal endothelium
- the endothelium, a neuroectoderm-derived cell layer composed of hexagonal cells arranged in a honeycomb-like mosaic pattern. The endothelial cells contain a high density of Na<sup>+</sup>, K<sup>+</sup>-adenosine triphosphatase (ATPase) pump sites contributing to corneal dehydration and fluid content regulation.

Inflammatory processes involving the cornea, whether infectious or non-infectious, are termed "keratitis." The corneal surface is normally protected by a variety of mechanisms including the physical barrier of eyelids to foreign material, regular blink response which sweeps away debris from the tear lake, and tight junctions between conjunctival and corneal epithelial cells. Immune mediators also protect the corneal surface: conjunctival mast cells, conjunctiva-associated lymphoid tissue (CALT) which is responsible for local antigen processing, immunoactive substances in the tear film (IgA, lysozyme,  $\beta$ -lysine, lactoferrin, and tear-specific albumin), plasma cells, macrophages and T lymphocytes. In the majority of keratitis cases, at least one risk factor that compromises these defense mechanisms can be identified.



**Figure 30-4** Normal cornea consists of five distinct layers. Epithelium (EP), Bowman's layer (BL), stroma (ST), Descemet's membrane (DM), and endothelium (EN).

The World Health Organization (WHO) has recognized corneal blindness resulting from infectious keratitis as an important cause of worldwide visual disability.<sup>165</sup> Risk factors for infectious keratitis include trauma (generally with plant or organic material), immunocompromised state, chronic ocular surface disease, and contact lens wear.<sup>56-58</sup> In most cases of infectious keratitis, a defect in the epithelium is the initial event predisposing to infection. The inflammatory response leads to cellular infiltration with destruction of the corneal collagen, thinning of the stroma and, in severe cases, perforation of the cornea with leakage of the aqueous humor and risk of intraocular extension, i.e., endophthalmitis.

### Epidemiology

Mycotic keratitis is relatively infrequent in the developed world but constitutes a large proportion of corneal infections in developing countries.<sup>59</sup> The clinical frequency and causative agent of fungal keratitis are influenced by geographic area. In general, fungal infections of the cornea are more common in warmer climates. In a report from Burma, two-thirds of all corneal ulcers were caused by fungi.<sup>60</sup> In a report from India, half of all culture-positive corneal ulcers yielded fungi.<sup>61</sup> In Florida, 35% of cases of keratitis were caused by fungi, with *Fusarium* being the most common clinical isolate.<sup>56,62</sup> In contrast, in New York, 1% of cases of keratitis have a fungal etiology, with *Candida* reported as the most common clinical isolate.<sup>63</sup>

Eye trauma is the overwhelming risk factor for mycotic keratitis, reported in 44–55% of affected patients.<sup>64</sup> Host factors that predispose to mycotic keratitis include epithelial or



stromal ulceration, lid margin notches, lagophthalmos, impaired tear secretion, reduced secretion of IgA in tears, and general immunosuppression. Lid margin notching or eyelid irregularities can lead to focal areas of surface epithelium desiccation and breakdown; likewise, lagophthalmos, the inability to completely close the eyelids, can predispose the unprotected cornea to surface breakdown. Lagophthalmos is a common finding in facial nerve paresis such as Bell's palsy and in neuromuscular diseases which lead to weakness of the orbicularis oculi muscle, the muscle which closes the eyelids. Fungal corneal ulceration has also been reported in patients with AIDS.<sup>65</sup>

Five percent of all infectious keratitis following refractive keratotomy such as LASIK is secondary to fungal infection.<sup>66</sup> With an increase in popularity of these procedures, a proportionate increase in fungal keratitis has been seen in this otherwise healthy patient population. Patients who use extended-wear contact lenses are at increased risk for all forms of keratitis, including those of fungal etiology.<sup>67</sup> An outbreak of fungal keratitis was attributed to contaminated contact lens cleaning solutions leading to a worldwide recall of this over-the-counter product.<sup>68</sup> Although contact lens wearers are typically young and healthy, the hypoxia and surface abrasive effect from the contact lens can compromise the corneal epithelium, thus increasing the risk of keratitis.<sup>69</sup> Fungal etiology should always be suspected when patients with presumed infectious keratitis do not respond to topical antibacterial agents.

## Pathogenesis

The pathogenesis of fungal keratitis is that of an opportunistic invasion of a compromised eye or an eye traumatized by organic matter. The inflammatory reaction and tissue destruction in fungal keratitis are caused by antigenic fungal cellular components, mycotoxins, and fungal proteases assisting in deeper stromal invasion.<sup>70</sup>

Species-specific factors influencing pathogenesis include adherence, invasiveness, morphogenesis, and toxigenicity. Invasiveness refers to the relative ease with which the fungus penetrates the corneal stroma and Descemet's membrane, allowing entry into the anterior chamber. Morphogenesis allows phenotypic switching, permitting fungi to survive different microenvironments in the infected host.<sup>71</sup> Although the role of fungal enzymes in keratitis remains debatable,<sup>72</sup> there is suggestion that fungal proteinases do play a role in the pathogenesis of keratitis.<sup>73,74</sup>

The progression of keratitis is highly variable, ranging from an indolent corneal ulcer in a contact lens wearer to a rapidly invasive infection resulting from traumatic exposure to organic matter. Intraocular invasion with loss of vision is the most dreaded consequence of fungal keratitis.

## Mycology

### Hyaline filamentous fungi

Filamentous fungi are the principal causes of mycotic keratitis in most parts of the world, with *Fusarium* and *Aspergillus* most commonly encountered. Filamentous fungal keratitis appears to occur most commonly in healthy young men engaged in agricultural work or outdoor occupations.<sup>56,74</sup>

Various traumatic agents such as vegetable matter, mud, paddy grain, and metallic foreign bodies have been reported as

risk factors for developing filamentous mycotic keratitis.<sup>56,74</sup> In addition, ocular trauma associated with nylon line lawn trimmers has also been associated with fungal keratitis.<sup>75</sup> Clearly, the unifying theme of risk is foreign material, either organic in composition or carrying organic matter, violating the cornea.

*Fusarium* spp. are the most frequently encountered etiologic agents of keratitis, particularly in tropical or subtropical regions.<sup>56,62,76</sup> *Fusarium* can invade the anterior chamber and form a lens-iris-fungus mass at the pupil, interfering with aqueous humor drainage and leading to elevation of the intraocular pressure.<sup>77,78</sup>

Contact lenses are now a major risk factor for keratitis. Measures to sanitize lens, including safe handling, storage, and cleaning, need to be emphasized to all patients. Contact lens wearers should wash their hands with soap and water, and then dry their hands before handling lenses, and wear lenses according to the schedule prescribed by their eyecare practitioners. Overwear and/or extended, continuous wear of contact lens has been shown to increase the risk of all forms of keratitis, so this practice should be discouraged to avoid potential visual damage from keratitis.

Furthermore, the recent rise in fungal keratitis among contact lens wearers may be attributed to the use of multi-purpose cleaning solutions. Contact lens solutions are generally formulated to be effective against bacteria. However, little attention has been given to their efficacy against fungi.<sup>79,80</sup> Small changes in composition of these solutions can greatly affect their efficacy. For example, a reduction in the concentration of biguanide from 0.0001% to 0.00005% is associated with a 10-fold increase in fungal contamination.<sup>79,81</sup> As mentioned, a worldwide outbreak of *Fusarium* keratitis occurred in soft contact lens wearers using a contaminated cleaning solution from one manufacturer.<sup>68</sup>

### Dematiaceous fungi

The dematiaceous fungi are common soil and plant saprophytes categorized on the basis of their dark pigment. Dematiaceous fungi are reported to be responsible for 10–15% of all fungal keratitis and are the third most frequently encountered fungi following *Aspergillus* and *Fusarium*.<sup>56,61,63,74,82–85</sup> Members of the dematiaceous fungi that have been recovered from infected corneas include species of *Curvularia*, *Exophiala*, *Exserohilum*, *Fonsecaea*, *Lecythophora*, *Phialophora*, *Scedosporium* and *Lasioidiplodia*. *Lasioidiplodia*, a cause of rot in fruit and vegetables, causes an especially severe form of keratitis.<sup>86–88</sup>

### *Candida*

*Candida* spp., predominantly *C. albicans*, usually occur in immunosuppressed patients, those with ocular surface disease or lid margin defects, or those receiving long-term topical corticosteroids.<sup>56</sup>

## Clinical manifestations

Symptoms of fungal keratitis include ocular pain, redness, diminished vision, photophobia, tearing, and discharge. On gross examination, the eye appears infected, and the cornea may have a noticeable haze, loss of luster or an area of opacification. A mucopurulent discharge may be present. The eyelids may be erythematous and edematous; reactive blepharospasm can make examination of the eye difficult.

Early signs of fungal keratitis, as observed by slit lamp biomicroscopy, include fine to coarse granular infiltrates in the anterior corneal stroma, feathery branching of the fungi into the stroma, and inflammatory cells and proteins in the aqueous humor. Although these signs are by no means universal in fungal keratitis, their recognition by the ophthalmologist should increase the index of suspicion of a fungal cause. Later corneal findings include an immune ring that can form focally in the corneal stroma around the infection, satellite lesions, and an endothelial plaque. Biomicroscopic features correlate well with histopathologic findings; hyphae of filamentary fungi tend to organize in the plane of the stromal lamella, and inflammatory cells migrate toward the organism (Fig. 30-5).

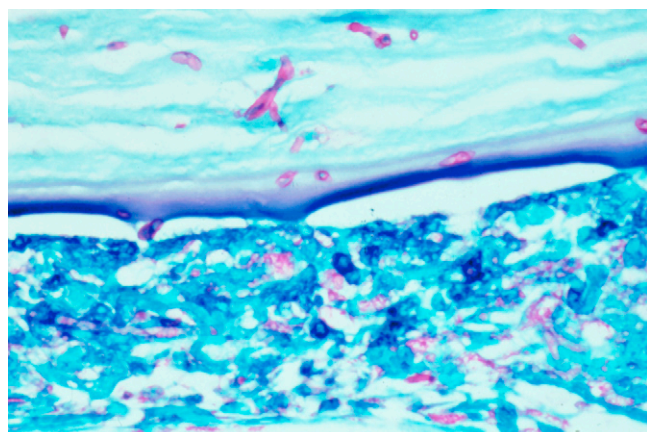
Keratitis caused by the dematiaceous fungi (*Curvularia* spp., *Exserohilum* spp.) can present as a persistent, low-grade keratitis with minimal structural alteration. Simple debridement of the pigmented necrotic tissue has been demonstrated to be sufficient treatment in some cases.<sup>82</sup> Keratitis caused by

*C. albicans* can resemble bacterial keratitis, with an epithelial defect, discrete infiltrate, and slow progression.<sup>89</sup>

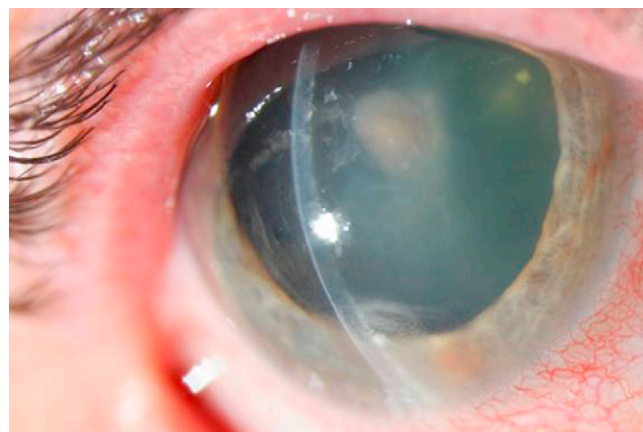
In advanced fungal keratitis the cornea becomes white (Figs 30-6 to 30-8), resembling bacterial keratitis, and corneal perforation through necrosis and ulceration may ensue. Endophthalmitis can be the consequence of corneal perforation and intraocular invasion.

## Diagnosis

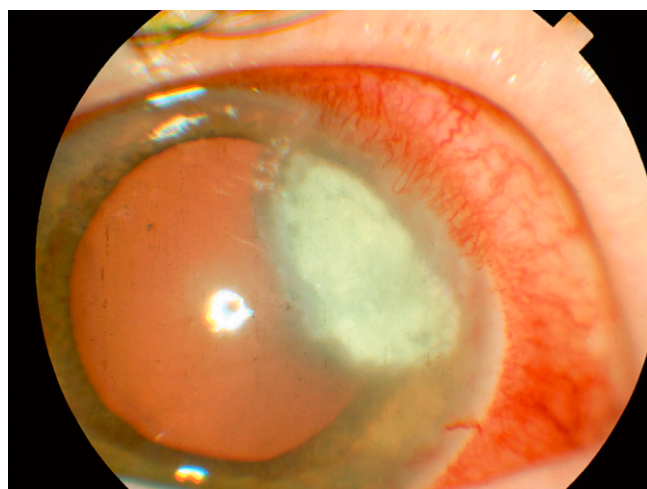
Diagnosis should be aggressively pursued with cultures and/or scrapings of the involved cornea using a platinum spatula, surgical blades or calcium alginate swabs. Corneal tissue specimens should be inoculated on the surface of solid media by making rows of "C" shapes and in liquid media by introducing the tip of the spatula into the broth several times. Most fungi will be visible in culture within 2–7 days, but several weeks may be required for definitive identification.



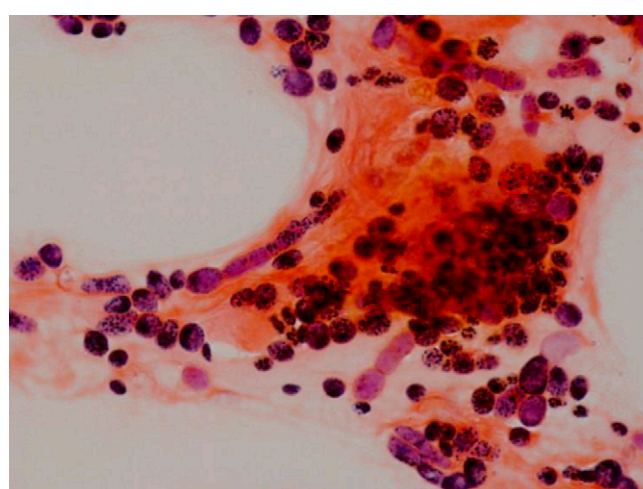
**Figure 30-5** Corneal button of a patient with fungal keratitis. Note the fungal elements and inflammatory processes in the cornea.



**Figure 30-7** Fifty five year old horsebreeder and contact lens wearer presented with fungal corneal ulcer. Note endothelial plaque on slit beam image (courtesy of Dr Joseph D. Iuorno).



**Figure 30-6** Fungal corneal ulcer. Stromal infiltration with feathery borders.



**Figure 30-8** Fungal Gram stain of the same patient as Fig. 30-7 with *Phaeoannellomyces werneckii* keratitis; infection resolved with topical amphotericin (courtesy of Dr Joseph D. Iuorno).

Corneal biopsy may be performed for patients in whom empiric antiinfective therapy has been unsuccessful and microbiologic diagnosis has not been established. Corneal biopsy can be performed at the slit lamp using a disposable 2 mm dermatologic punch.<sup>90</sup> Several studies have highlighted the value of corneal biopsy in diagnosing fungal keratitis when conventional corneal scrapings do not yield positive results.<sup>91,92</sup>

The fungi are easily visualized with standard tissue stains; however, the Fontana–Masson stain is most useful for detecting dematiaceous fungi because of the melanin in the fungal wall. Confocal microscopy, which allows optical sectioning, is a non-invasive technique and may be useful in identification of corneal pathogens in the early stages, when this instrument is available.

In patients who undergo penetrating keratoplasty secondary to infectious corneal perforation, the diseased corneal tissue should be studied microbiologically and histopathologically as the results can guide postoperative management and may assist in predicting surgical outcome. Fungal structures in corneal specimens may be stained with PAS and GMS techniques. The inflammatory reaction seen in mycotic keratitis is less severe than bacterial keratitis and usually consists of lymphocytes and plasma cells.<sup>93</sup> There may be coagulative necrosis of the corneal stroma with “satellite” microabscesses.<sup>93</sup>

## Treatment

Natamycin 5% suspension administered topically is widely used for suspected fungal keratitis.<sup>94</sup> Natamycin is administered to the infected eye every hour, all day for 1 week and then every hour during the day while awake for 12 weeks.<sup>94,95</sup> Large ulcers and *Aspergillus* infection are less likely to respond favorably to monotherapy with topical natamycin.<sup>96</sup> Amphotericin B 0.15% solution alone or in combination with natamycin 5% or flucytosine 1% has been advocated by some authors as empiric treatment of severe, large ulcers, suspected of harboring fungus.<sup>94,95</sup> As always, treatment should be modified based on clinical response and once the offending organism has been identified from culture.

Topical natamycin combined with oral itraconazole or oral ketoconazole is widely used for confirmed *Aspergillus* keratitis.<sup>85,160</sup> Amphotericin B 0.15% solution has been widely used for confirmed *Candida* and cryptococcal keratitis.<sup>56</sup> For fungal keratitis due to dematiaceous fungi, natamycin alone or in combination with topical clotrimazole or topical miconazole has been shown to be effective in 88% of cases.<sup>82</sup> Topical miconazole or ketoconazole has also been reported to be efficacious against these pathogens.<sup>39</sup> However, in patients with dematiaceous keratitis with deeper tissue invasion, addition of oral ketoconazole is recommended.<sup>82</sup> Subconjunctival injection of miconazole has been used when threatening ocular perforation or endophthalmitis exists. In severe cases systemic therapy with ketoconazole (400 mg/day) or itraconazole (400 mg/day) is advised.<sup>94,95</sup> Posaconazole has proven effective in anecdotal cases when other azoles and amphotericin therapy have failed.<sup>97-99</sup>

Adjunctive treatment with mydriatics to prevent posterior synechiae formation (iris to lens adhesions) and cycloplegics to reduce ciliary spasm is indicated when anterior chamber inflammation is present. Many clinicians would empirically

treat with cycloplegics as ciliary spasm can induce incapacitating pain. Narcotic analgesics and oral antiinflammatories are frequently provided for patient relief.

The use of topical corticosteroids is controversial; some authors feel corticosteroids are contraindicated for the management of all fungal keratitis.<sup>100,101</sup> However, if topical corticosteroids are used, they should only be administered after 7–10 days of antifungal therapy, with unequivocal clinical improvement and close interval examination.

Debridement of the infected corneal tissue followed by coverage with a conjunctival flap, along with concomitant antifungal therapy, has been advocated for small non-healing peripheral ulcers.<sup>56</sup> Amniotic membrane grafting has also been used as an effective means of promoting reepithelialization and preventing perforation in acute fungal keratitis.<sup>102</sup> This tissue is commercially available in dehydrated form.

If the inflammatory reaction or infection leads to corneal necrosis with actual or impending perforation, full-thickness corneal grafting (penetrating keratoplasty) may be necessary. Studies have shown that fungal keratitis is associated with a sixfold higher risk of perforation and need for penetrating keratoplasty, compared to bacterial keratitis.<sup>76</sup> Penetrating keratoplasty is performed in 15–28% of patients with mycotic keratitis who have failed medical therapy.<sup>82</sup> During keratoplasty surgery, the lens should not be disturbed if possible so as to reduce the risk of intraocular infection.<sup>103</sup>

Graft failure due to recurrent infection is of major concern. Up to 95% of the corneal graft failures have been reported, most occurring in the first month and due to a combination of infection and/or rejection.<sup>104</sup> Cautious use of postoperative topical corticosteroids and topical 0.5% cyclosporine A may reduce the rate of graft rejection.<sup>105</sup>

## Sinoorbital disease

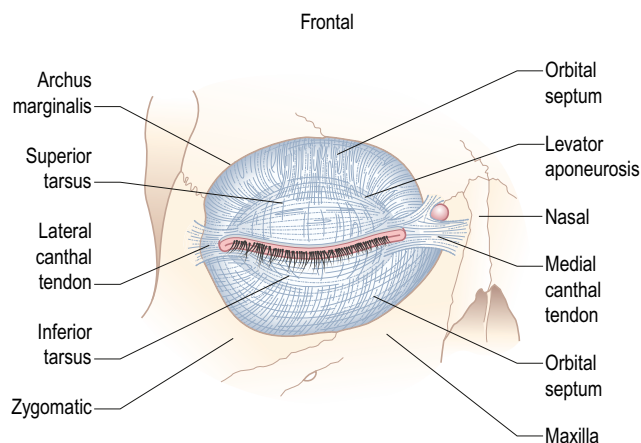
### Anatomy

The orbital septum is an important anatomic barrier in the eyelids that prevents contiguous spread of infection posteriorly from the eyelids into the orbital tissues. The septum is a thin fibrous layer arising from the periosteum along the inferior and superior orbital rims which fuses into the upper and lower eyelid retractors (Fig. 30-9). By definition, tissues anterior to the septum are part of the eyelids and tissues posterior to the septum are in the orbit. Infectious processes in the orbit can rapidly spread to involve extraocular muscles and cranial nerves. Any mass effect from orbital infection or inflammation can lead to proptosis, an anterior displacement of the eye. Most orbital infectious problems arise from the sinuses by contiguous spread. Unlike preseptal infections involving the eyelids, which are predominantly caused by Gram-positive bacteria, orbital infections are often caused by fungi or a mixed bacterial etiology.

### Epidemiology

The vast majority of orbital fungal infections are secondary to contiguous spread from infected paranasal sinuses.<sup>106,107</sup> Less often, traumatic implantation of contaminated foreign bodies or hematogenous seeding has been incriminated.





**Figure 30-9** Normal anatomy of the eyelid.

Immunocompromised patients, those receiving chemotherapy, chronic corticosteroids or immunosuppressive agents, intravenous drug abusers, and neutropenic patients are all at increased risk for invasive fungal infections of the orbit. Fungal orbital infection in immunocompetent individuals is usually associated with antibiotic use or breakdown of mucocutaneous barriers.<sup>108</sup>

## Mycology

### *Aspergillus*

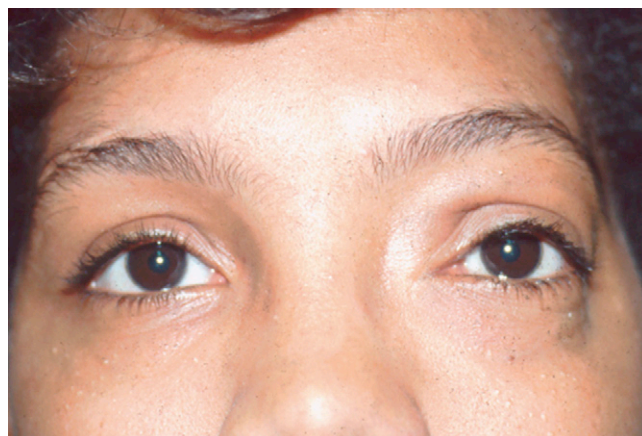
Different presentations of *Aspergillus* infection of the orbit may occur even in a healthy host. Aspergillosis is the most common fungal sinus infection in immunocompetent patients, causing an indolent, chronic infection with granulomatous host response, i.e., aspergilloma (Fig. 30-10). In contrast, aspergillosis in immunocompromised hosts usually presents fulminantly without granuloma formation. In one study, 92% of patients with invasive *Aspergillus* involving the orbit had some form of malignancy.<sup>109</sup> Fifty percent of orbital infections in HIV patients are caused by *Aspergillus*,<sup>110</sup> *A. flavus* being the most common species implicated.

### Zygomycosis

Rhino-orbital-cerebral zygomycosis represents the prototype of fulminant, invasive orbital fungal infections with an acute course in the immunocompromised host. *Rhizopus* and *Rhizomucor* are the most common fungi causing zygomycosis. Other species that have been implicated in immunosuppressed hosts are *Absidia*, *Mucor*, *Cunninghamella*, *Saksenaea*, and *Apophysomyces*.<sup>111-113</sup> *Apophysomyces elegans* is an emerging cause of zygomycosis in immunocompetent patients following traumatic inoculation.<sup>111</sup>

Zygomycosis originates in the nasal/sinus mucosa. After proliferation in the nasal cavity, the zygomycete reaches the pterygopalatine fossa, inferior orbital fissure and finally the retroorbital space, resulting in ophthalmic signs and symptoms. Angioinvasion and marked tissue necrosis are the key features of this fungal infection which is responsible for high rates of morbidity and mortality.

Diabetic ketoacidosis is the most common predisposing factor (60–80%).<sup>114</sup> However, invasive zygomycosis has



**Figure 30-10** Sinus aspergilloma with orbital extension. Note the proptosis and temporal displacement of the left eye.

also been associated with other immunocompromised states, including the use of immunosuppressive medications following organ transplantation and neutropenia. Zygomycosis may be seen following deferoxamine treatment for iron/aluminum overload.<sup>115</sup> Broad-spectrum antibacterial therapy has been reported as a risk factor for zygomycosis.<sup>114</sup>

## Clinical manifestations

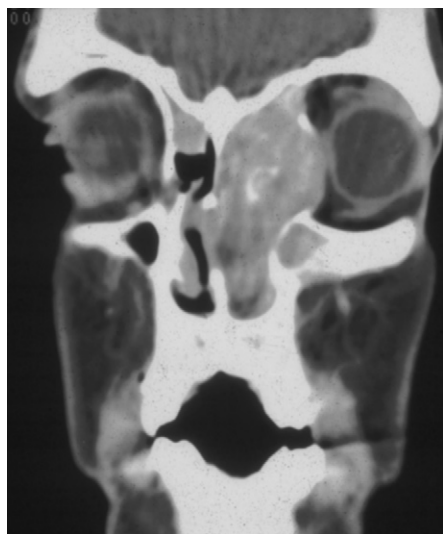
In immunocompetent patients, as mentioned previously, the course of the disease is often chronic. Facial heaviness or fullness and nasal discharge are often presenting complaints. With orbital involvement, non-axial globe displacement and/or proptosis are seen (Fig. 30-11), but typically without evidence of optic nerve compromise or other cranial nerve dysfunction.

In contrast, immunocompromised patients with invasive fungal sinoorbital disease typically present with facial pain, headache, or other symptoms of fulminant sinusitis. With more advanced disease and extension into the orbit, proptosis, diplopia secondary to extraocular muscle dysfunction and/or cranial nerve paresis, and decreased vision are the usual presenting ophthalmic findings. With involvement of the orbital apex and invasion of cranial nerves II, III, IV, V, or VI, the patient demonstrates complete or partial external ophthalmoplegia, upper facial anesthesia, and vision loss. Visual loss is a result of invasion of CN II and/or thrombosis of the central retinal artery.<sup>116</sup> Facial nerve involvement indicates more extensive disease outside the retroorbital space and is a grave prognostic indicator.

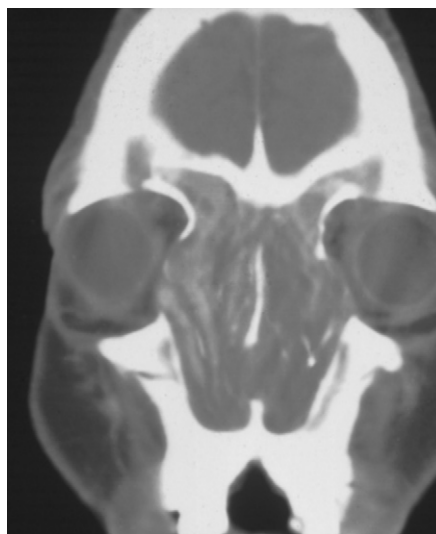
In rhino-orbital-cerebral zygomycosis, the affinity of the infecting organism for blood vessels leads to arterial thrombosis, necrosis, and infarction. Thick, dark nasal discharge is seen, with the hallmark black necrotic turbinates and nasal septum. Although tissue necrosis is considered a classic feature, its absence should not preclude the diagnosis. In one study, only 19% of cases demonstrated tissue necrosis at the time of presentation.<sup>116</sup> Nevertheless, turbinate or septal necrosis is a highly predictive clinical sign for zygomycosis.

The infection has a tendency to spread along the nerves, ophthalmic artery or cribriform plate to the meninges or the brain.<sup>117</sup> Necrosis and infarction of the brain may occur because of vascular invasion, and the disease may prove to





**Figure 30-11** Coronal CT image of patient in Fig. 30-10. Note calcification in maxillary-ethmoid mass highly suggestive of aspergilloma.



**Figure 30-12** Coronal CT image of a patient with bilateral aspergilloma with extension into the orbits.

be rapidly fatal. Bilateral cavernous sinus thrombosis, isolated pontine infarction, palatal ulcer, sudden blindness, fever with hemiparesis, and dysarthria have all been reported as manifestations of zygomycosis.<sup>118-120</sup> However, it must be remembered that rhino-orbital-cerebral zygomycosis may manifest as a painless orbital apex syndrome without signs of sinusitis, orbital cellulitis or acute systemic disease.<sup>81</sup>

## Diagnosis

Radiographic imaging of the orbit and paranasal sinuses is invaluable for both the initial evaluation and for monitoring disease progression and response to treatment (Figs 30-12, 30-13). In addition, detailed radiologic study is mandatory for surgical planning. Both computed tomography (CT) and magnetic resonance imaging (MRI) play a role in defining the extent of the infectious process. MRI provides details of the soft tissue anatomy of the orbit and intracranial structures superior to those of CT. Also MR with contrast can provide vascular images, including the carotid and cavernous sinus. On the other hand, CT provides superior imaging of bone and fungal mass within the sinuses. Both axial and coronal high-resolution images by CT or MRI should be obtained (see Figs 30-10 to 30-13). Coronal images can be obtained by MR without special positioning of the head, which may be advantageous in obtunded or non-cooperative patients. In contrast, CT coronal images require neck flexion, which is not always feasible in severely ill patients. CT and MRI often provide complementary information, and patients should be evaluated by both techniques; subtle findings on MR may not be seen by CT and vice versa.

Often bedside nasal debridement can provide an adequate tissue specimen for study, avoiding more invasive procedures. Samples can be obtained from the septum, lesions on mucus membranes, material aspirated from sinuses, bronchial washings, or aspirated material from abscesses. Multiple biopsies should be taken.<sup>121</sup> Once collected, samples should be taken to the laboratory immediately due to the fragility of zygomycetes, which do not survive more than a few hours at refrigerator

temperature. The microscopic demonstration of zygomycetes in KOH mounts or stained smears is more significant than their isolation in culture.<sup>113,121</sup> Zygomycetes frequently do not grow in cultures from necrotic tissue. Culture media should be inoculated with as large a specimen as is feasible. In vitro susceptibility testing should be performed to exclude amphotericin resistance.<sup>122</sup>

An enzyme-linked immunosorbent assay may be used to demonstrate antibodies to the specific etiologic agent. This method has not gained widespread use, most likely secondary to the fulminant course of most rhino-orbital-cerebral zygomycosis and more direct methods for diagnosis, i.e., staining and culture of involved tissues.

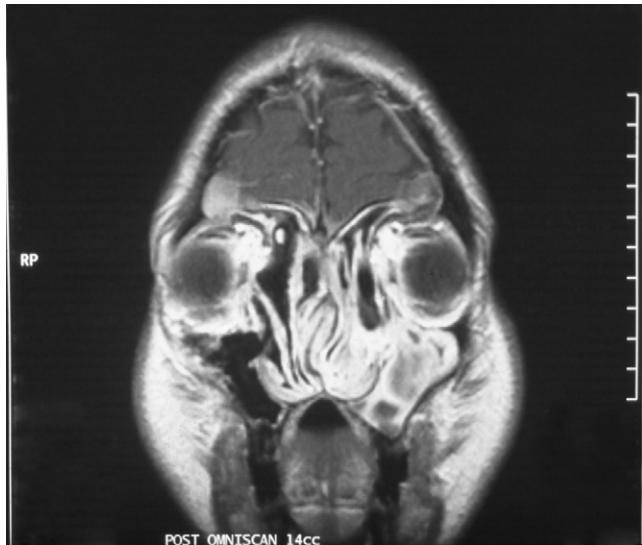
## Treatment

The treatment of fungal sinoorbital infection is combined surgical debridement and antifungal therapy. Prompt recognition and treatment are essential for halting the progression of the disease and preventing death. A review of patients reported in the literature between 1970 and 1994 revealed that 81% survived when the interval between the onset of symptoms and surgery was 1–6 days, compared to 52% when the interval was 7–12 days and, 42% when the interval was 13–30 days.<sup>114</sup>

Surgical debridement of all necrotic tissue is crucial, and often requires multiple surgeries. Wide local excision of all involved and devitalized tissue is required. Frozen section guided surgical debridement techniques for biopsy-proven zygomycosis have been advocated.<sup>123</sup> An external or transantral approach used to be the classic method for surgical debridement. However, endoscopic sinus surgery has been implemented for radical resection with excellent survival.<sup>124</sup>

Orbital exenteration was once considered mandatory in the presence of orbital involvement but clearly can be avoided in many cases.<sup>125</sup> Orbital exenteration is a consideration in the setting of an acute infection when the eye is blind.<sup>114</sup>

Antifungal therapy is a vital adjunctive to surgery and essential to a successful outcome. Aggressive surgical debridement



**Figure 30-13** MR image of the same patient shown in Fig. 30-10. More soft tissue details can be viewed as compared to CT (Fig. 30-11).

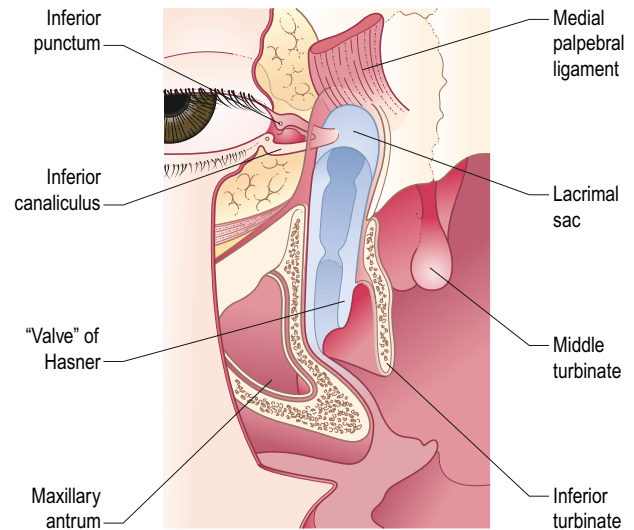
combined with intravenous amphotericin B remains the mainstay for invasive zygomycosis. Timing of intravenous amphotericin B is crucial to outcome: when the treatment was started within 1–6 days of symptom onset, 76% of patients survived, compared to 36% when the interval was 7–30 days.<sup>114</sup> The initial dose in a critically ill patient is 1–1.5 mg/kg/day for the first several days. A lower maintenance dose of 0.8–1 mg/kg/day is used after several days.<sup>122</sup> Once a response is documented by clinical and radiographic evaluation, the dose can be given every other day to minimize toxicity. The liposomal forms of amphotericin B may be more readily tolerated. Direct delivery of amphotericin B to the infected tissue by daily irrigation and packing of the involved orbit and/or sinuses with amphotericin combined with intravenous amphotericin B resulted in excellent outcomes in a small series of patients.<sup>125</sup> Both itraconazole and fluconazole have been used successfully in anecdotal reports of rhinocerebral zygomycosis.<sup>126,127</sup> Posaconazole has been shown to be effective in treating zygomycosis. Hyperbaric oxygen has been advocated as adjunctive therapy with success.<sup>114,126–129</sup> Successful use of interferon- $\gamma$  as adjunctive therapy has been reported anecdotally.<sup>122</sup>

The prognosis of rhino-orbital-cerebral zygomycosis was poor in the not so distant past, with mortality rates as high as 90%. Fortunately, mortality has declined to 15–35%, because of earlier diagnosis, high-resolution imaging and more rapid and aggressive treatment plans.<sup>130</sup> Factors contributing to a lower survival rate include delayed diagnosis and treatment, bilateral sinus involvement, leukemia, renal disease, history of treatment with deferoxamine, hemiparesis or hemiplegia.<sup>114</sup>

## Dacryocystitis and canaliculitis

### Anatomy

The lacrimal outflow system begins with the pinpoint opening, the punctum, in the medial upper and lower eyelids (Fig. 30-14). The superior and inferior puncta are the proximal



**Figure 30-14** Normal anatomy of lacrimal excretory system.

openings of the respective superior and inferior canaliculi, delicate duct structures intimate with the medial canthal tendon. The upper and lower canaliculi merge, in most individuals, into a short common canaliculus before entering the lacrimal sac. Tears exit from the sac down the nasolacrimal duct and empty into the nasal passage in the inferior meatus. The constant contraction and relaxation of the orbicularis oculi during normal blinking account for the intraluminal pressure changes that move the tears from the eye into the nose. An anatomic obstruction in the lacrimal outflow system, usually in the nasolacrimal duct, predisposes the patient to tear stasis and infection of the lacrimal sac, also known as dacryocystitis.<sup>131</sup> Dacryocystitis is the most common infection of the lacrimal apparatus.

### Epidemiology

Fungal dacryocystitis accounts for 5% of all acquired dacryocystitis, and 14% of cases of congenital dacryocystitis.<sup>132–134</sup> Women are affected more frequently with all forms of dacryocystitis than men, probably because of anatomically narrower nasolacrimal ducts.<sup>128</sup> It most commonly affects individuals in their 50s or 60s. Recent or past midfacial trauma, particularly nasoethmoid fracture, predisposes the patient to nasolacrimal obstruction and dacryocystitis. Other risk factors include dacryolith formation, and nasal or paranasal sinus disease. Allergy or chronic inflammation of the nasal mucosa impedes outflow from the duct, worsening stasis and increasing the risk of dacryocystitis.

### Mycology

Fungi implicated in dacryocystitis include species of *Acremonium*, *Aspergillus*, *Candida*, *Paecilomyces*, *Rhizopus*, and dermatophytes. *Candida albicans* and *A. niger* are the fungi most frequently isolated.<sup>133,135</sup> *Aspergillus*, *Candida*, *Paecilomyces*, *Rhinosporidium seeberi*, and dermatophytes can cause a chronic granulomatous dacryocystitis.<sup>136,137</sup>

## Clinical manifestations

Dacryocystitis typically presents with erythema, induration, and sensation of pressure in the medial canthus. Because of retrograde regurgitation of the infected matter from the lacrimal sac to the ocular cul-de-sac, the eye may be red and the eyelids edematous. Preseptal cellulitis may be seen particularly with rupture of a distended lacrimal sac (Fig. 30-15). Fistula formation, when it occurs, usually involves the skin overlying the inferior medial orbit (Fig. 30-16).

Pain frequently is severe and may localize to the glabellar region due to irritation of the supratrochlear nerve. Dacryocystitis should be considered in patients presenting emergently with acute pain in the lower forehead, particularly with a history of tearing, fullness and/or tenderness in the medial canthus. However, the infection is more often indolent and the pain mild.

Canaliculitis presents with unilateral conjunctivitis, mucopurulent discharge from the puncta, pouting of the punctum, and focal inflammation over the involved canaliculus. It is not a common ocular adnexal infection and most often is caused by *Actinomyces* and infrequently by fungi.

## Treatment

Initial treatment of dacryocystitis is oral antimicrobial therapy. If the lacrimal sac is distended, needle aspiration with a 16 or 18-gauge needle can be used to eliminate the associated discomfort and prevent fistula formation, and the aspirate can be submitted for culture. Hospitalization is rarely necessary except in debilitated or pediatric patients. In patients with recurrent dacryocystitis or those not responding to oral therapy, surgical drainage of the infected sac combined with dacryocystorhinostomy is indicated. If the infection recurs after dacryocystorhinostomy, dacryocystectomy may be warranted, as a nidus of infection can persist in the sac or duct remnant or both. One study demonstrated notably better results with combined surgical and medical treatment, with an 80% cure rate, versus medical treatment alone, with a 10% cure rate.<sup>138</sup> Infants are typically treated by probing of the lacrimal duct, sometimes in conjunction with silicone intubation of the lacrimal system to maintain patency.

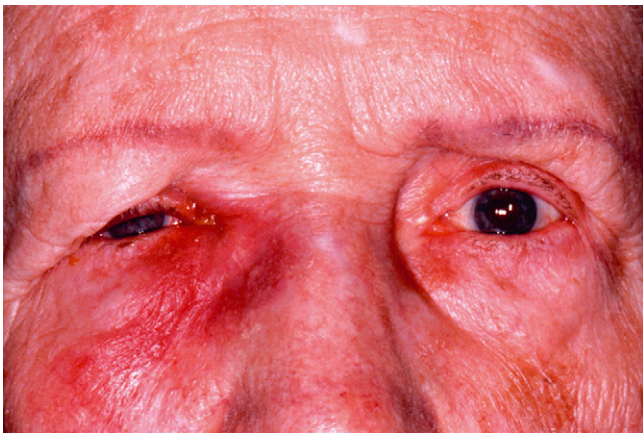
## Ocular histoplasmosis syndrome or presumed ocular histoplasmosis syndrome

### Epidemiology

Presumed ocular histoplasmosis syndrome (POHS) is an inflammatory syndrome that has been associated with systemic clinical and subclinical infection by *Histoplasma capsulatum*. Although the overall scientific evidence is questionable that this syndrome represents *Histoplasma* infection, it is mentioned because of the link to *Histoplasma* exposure and its historic significance.

This clinical syndrome is most commonly seen in the Ohio and Mississippi River valleys of the United States. In areas endemic with *H. capsulatum*, 59% of the population has evidence of exposure by skin testing to *Histoplasma*, but only 4.4% of skin test-positive individuals have characteristic POHS fundus lesions.<sup>139, 140</sup> Although the typical POHS findings are most frequently observed in patients inhabiting or having traveled to areas of the United States in which histoplasmosis is endemic, identical ocular findings have been described in patients from areas of the world without known histoplasmosis.<sup>161</sup> For example, in The Netherlands, serologic testing for *H. capsulatum* was negative for all patients with clinical POHS.<sup>164</sup> It may be that the ocular findings in POHS are not caused exclusively by *H. capsulatum* but are a result of a common pathologic process with an identical ophthalmic picture. There is no conclusive evidence of systemic histoplasmosis in POHS patients, which is in contradistinction to known cases of *Histoplasma* uveal infection where this is part of a disseminated disease picture.<sup>141</sup>

Ocular histoplasmosis is most commonly seen in middle-aged people and more frequently in whites. Two HLA antigens have been found to be associated with POHS: HLA-B7 and HLA-DRw2.<sup>142-144</sup> The HLA association suggests that POHS is part of a spectrum of autoimmune diseases triggered by an infectious organism, *H. capsulatum* being one of the several candidates.



**Figure 30-15** Preseptal cellulitis in a patient with dacryocystitis.



**Figure 30-16** Fistula formation in a case of chronic *Candida* dacryocystitis.



## Pathogenesis

Spencer et al<sup>161</sup> detected *H. capsulatum* DNA by laser capture microdissection and polymerase chain reaction from macular and mid-peripheral choroidal lesions in a pathology specimen with POHS but did not detect *H. capsulatum* DNA from uninvolved choroids. The authors suspected that POHS is a chronic reaction to the immunogenic residue of the organism. Histopathologic examination revealed mixed inflammatory cells in the choroid, loss of retinal pigment epithelium, and adhesions between outer retina and choroid.

## Clinical manifestations

Most patients with POHS are asymptomatic and the fundus changes are found on routine eye examination. Classically defined fundus changes include peripapillary atrophy, multiple atrophic choroidal scars with central hypopigmentation and peripheral hyperpigmentation, with complete absence of any signs of vitreal inflammation (Fig. 30-17).

Peripapillary atrophy is seen as loss of the choroid and retina surrounding the optic disk. The choroidal lesions, which are usually sharply circumscribed, are referred to as “histo spots” and are the hallmark of the syndrome. Clarity of the vitreous implies that the pathologic process in POHS does not extend into the vitreous. Vitreous clarity also helps differentiate POHS from other similar chorioretinal disease processes. Histo spots are found bilaterally in 62% of POHS patients.<sup>145</sup> New histo spots develop in more than 20% of individuals while they are under observation.<sup>146</sup> Histo spots near the macula increase the risk for the development of choroidal neovascularization (CNV). Macular CNV may cause loss of central visual acuity or metamorphopsia, i.e., distortion of visual images. The events leading to the development of CNV in POHS have not been defined but disruption of Bruch’s membrane likely plays a role in its development.

Microscopic analysis of the submacular CNV demonstrated fibrovascular tissue interposed between the Bruch’s membrane

and the retinal pigment epithelium.<sup>147</sup> Cellular components in the neovascular membrane include retinal pigment epithelium, vascular endothelium, photoreceptors, and mixed inflammatory cells. Subretinal neovascular membranes can leak fluid and serum proteins, leading to serous or hemorrhagic retinal detachment and vision loss and/or distortion. The neovascular membrane may resolve into a disciform scar with subretinal fibrosis.

*Histoplasma* endophthalmitis has been reported but is rare and does not present with the fundus findings typically seen in POHS.<sup>148</sup>

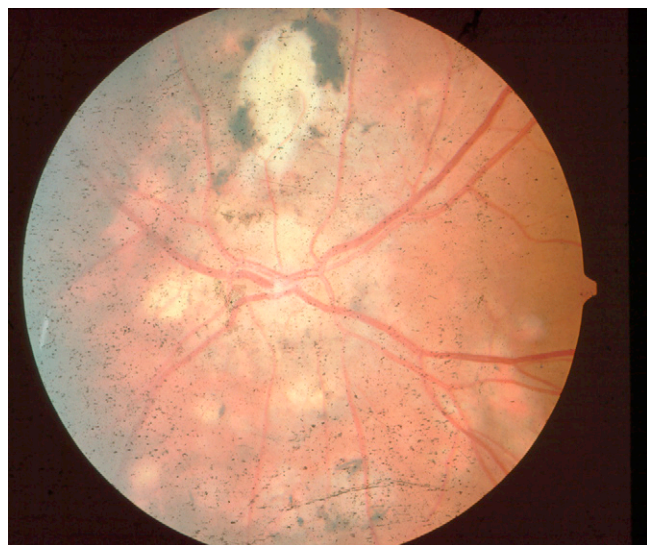
## Diagnosis

Diagnosis is made by the classic ophthalmologic findings described above. Once POHS has been identified in a patient, the principal concern is future development of a macular neovascular membrane. The incidence of these membranes is not well established because of the asymptomatic nature of the syndrome in most patients. Since neovascular membranes in the macula arise from histo spots in that area, patients identified with these findings should be viewed as “at risk” and monitored closely (Fig. 30-18).

Home monitoring for changes in the macula is done with the Amsler grid or a similar diagram. The Amsler grid is a graph paper checkerboard with a central dot. The patient is instructed to visually fixate on the dot using one eye at a time. If the patient notes a new visual defect in the checkerboard pattern, distortion or blurring of the lines, prompt examination is recommended to exclude recent development of a neovascular membrane.

## Treatment

Of the pathologic findings that constitute POHS, subfoveal and juxtafoveal neovascularization present the most significant clinical problem. Since there is no evidence of infection, antifungal therapy does not play a role in treatment. Therapy



**Figure 30-17** Presumed ocular histoplasmosis syndrome (POHS). Note the chorioretinal scarring, peripapillary atrophy, and absence of vitritis.



**Figure 30-18** Choroidal neovascular membrane in a patient with POHS involving the macula. Note peripapillary atrophy and chorioretinal lesions.



is directed at ablating POHS-related CNV. The Macular Photocoagulation Study showed a benefit in treating extrafoveal and juxtafoveal CNV associated with POHS by thermal laser treatment.<sup>149</sup> The fovea is the central area of retina conferring precise visual acuity.

In patients with juxtafoveal lesions, krypton laser treatment resulted in a 4.6% rate of severe visual loss versus 24.6% in the untreated group. The principal drawbacks to treatment with thermal laser are the resulting central scotoma and the incomplete treatment effect at the edge of the lesion, near the fovea. Given the immediate and profound visual loss that results from the laser-induced scotoma, laser photocoagulation is not used for treatment of subfoveal CNV.

Photodynamic therapy (PDT) with verteporfin (Visudyne, Novartis, Basel, Switzerland) is a two-step procedure, consisting of intravenous infusion of verteporfin, a photosensitive drug, which is then activated by a specific wavelength laser, resulting in incomplete occlusion of the treated vessels. The advantage of PDT is its selective damage to CNV without harming the overlying retina. In 2001, after publication of the 1-year results from the Verteporfin in Ocular Histoplasmosis Study,<sup>150</sup> the US Food and Drug Administration approved PDT for CNV secondary to POHS. The 2-year results of the study showed improvement of visual acuity from baseline.<sup>67</sup>

Intravitreal corticosteroids have been used for POHS choroiditis and have also been explored as a possible treatment for CNV. Molecular studies of neovascularization suggest that inflammatory cells participate in the neovascular response and corticosteroids may suppress this response.<sup>151</sup> Therefore, intravitreal corticosteroid therapy is being studied in combination with photodynamic therapy for CNV associated with POHS and other etiologies.

Vascular endothelial growth factor (VEGF) has been demonstrated in human subfoveal neovascular membranes, making it a promising target for CNV treatment. Several anti-VEGF treatments are currently being pursued for the treatment of CNV associated with age-related macular degeneration, and will likely be adopted for CNV associated with POHS. Anti-VEGF treatments are approved by the US Food and Drug Administration for intravitreal injection and treatment of neovascular membranes and include: 28-base ribonucleic acid aptamer pegaptanib (Macugen, Eyetech/Pfizer, New York, USA), and ranibizumab (Lucentis, Genentech, San Francisco, USA), an active fragment of a humanized anti-VEGF monoclonal antibody.

Bevacizumab is an anti-VEGF agent currently approved for the treatment of metastatic colorectal cancer. It has not been approved by the US FDA for intravitreal injections. However, employment of this agent in multiple clinical studies for the treatment of CNV has shown promising results. The main problem associated with anti-VEGF treatment is the need for multiple intravitreal injections and the associated risk of endophthalmitis.

Submacular surgery for the removal of CNV was first introduced in 1988. The main rationale for the surgery is based on the assumption that by limiting the size of the subfoveal lesion, the degree of visual acuity loss would be diminished. A multicenter randomized clinical trial suggested that surgery was advantageous to patients with visual acuity worse than 20/100.<sup>152</sup> Because of potential complications associated with this surgery, it is unclear whether benefits outweigh risks.

## Cryptococcus

### Epidemiology

The primary site of infection is the lung, but the disease often manifests in the central nervous system.<sup>153,154,162</sup> Ocular involvement occurs after cryptococcal meningitis and may represent hematogenous dissemination or extension through the leptomeninges. Visual loss is the most catastrophic complication, since it is often irreversible. Kestelyn found that 76% of patients with systemic cryptococcal disease had ocular findings.<sup>154</sup>

In Papua, New Guinea, *Cryptococcus neoformans* var. *gattii* causes 95% of the cases of cryptococcal meningitis; visual loss is common and may occur in immunocompetent patients. In contrast, in reported cases of meningitis due to *C. neoformans* var. *neoformans*, visual loss is rare and usually occurs in immunocompromised patients. It is not clear whether the *gattii* variant is more virulent or whether there is delayed diagnosis of patients from these tropical areas.

### Pathogenesis

The pathogenesis of visual loss in cryptococcal meningitis is uncertain, although it has been attributed by different authors to be due to: (1) blood-borne dissemination leading to chorioretinitis and endophthalmitis, (2) optic neuritis due to fungal spread from infected leptomeninges and/or (3) increase in intracranial pressure. Other suggested mechanisms include compression of the optic nerve by adhesions and cryptococcoma.<sup>33,155</sup> The chorioretinitis–endophthalmitis hypothesis does not account for many of the patients with profound visual loss as this diagnosis is not substantiated by clinical observation. It is interesting to note that one study found higher rates of visual loss from cryptococcal meningitis in immunocompetent patients;<sup>156</sup> this was theorized to be due to enhanced inflammatory response with compression damage to the optic nerve.

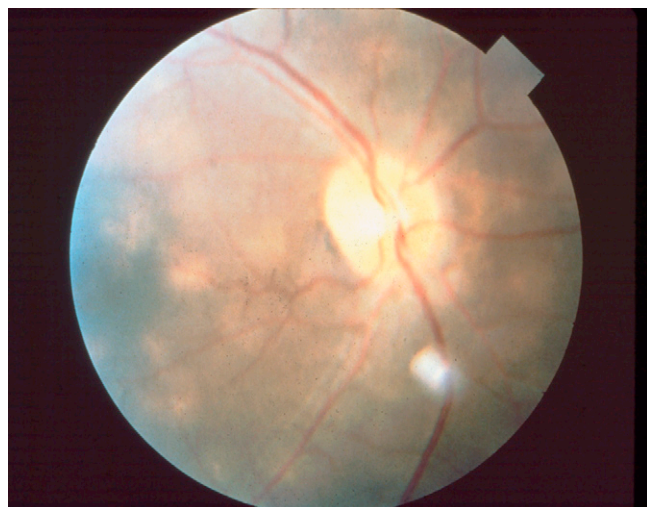
### Clinical manifestations

Two distinct clinical patterns of visual loss have been seen in patients with cryptococcal meningitis. The first pattern presents with rapid visual loss – visual deterioration in less than 3 days, which tends to be extreme and permanent. These cases are similar to the presentation of demyelinating optic neuritis; in one case, direct invasion of the optic nerve was shown pathologically. No effective treatment has been found for this group.

The second category presents with slow visual loss that tends to be mild and is often associated with transient visual obscurations or visual field defects. These findings are likely due to elevated intracranial pressure (ICP) associated with the meningitis. Papilledema is usually seen, supporting this mechanism as the cause of visual symptoms.

Other ophthalmic manifestations of cryptococcal meningitis include ophthalmoplegia, diplopia, nystagmus, sixth cranial nerve palsy, ptosis, optic atrophy, central vein occlusion, and cryptococcal choroiditis.<sup>157,158</sup>

The clinical features of cryptococcal choroiditis are cells in the vitreous with focal choroidal lesions (Fig. 30-19). The presence of organisms in the choroid implies hematogenous spread



**Figure 30-19** *Cryptococcus* chorioretinitis. The blurring of the image is due to the associated vitritis with loss of distinct fundus details.

and, consequently, is associated with poor prognosis. Other ocular findings include cotton wool spots, Roth spots, flame-shaped hemorrhages, perivascular sheathing, and microaneurysms. However, as mentioned earlier in the chapter, these are non-specific findings that may not be directly associated with the choroiditis, as they may be signs of other systemic diseases, including HIV infection. Fluorescein angiography and indocyanine green videoangiography can be employed to aid in diagnosis of cryptococcal choroiditis. Fluorescein angiography often shows a multifocal pattern of irregularly shaped hypofluorescent spots relating to active disease and choroidal infiltration.

## Treatment

The prognosis for AIDS patients with systemic cryptococcosis and choroiditis is poor.<sup>163</sup> Antifungal therapy for cryptococcal meningitis is described elsewhere in this book but amphotericin B, flucytosine, and fluconazole can be successfully used in various combinations and various routes of administration. Since the ophthalmic issues are typically part of the systemic picture, most patients are monitored for visual parameters but no ocular measures taken. Intravitreal amphotericin B in combination with systemic antifungal therapy has also been administered successfully.

Serial lumbar punctures are the modality most commonly used to decrease ICP, although no standardized protocol has been proposed. This approach often alleviates the clinical manifestations in patients with persistently high ICP. Lumbar drains and lumbar peritoneal shunts are successful in decreasing ICP when serial lumbar punctures have proven ineffective.<sup>159</sup> Optic nerve sheath fenestration has been demonstrated to provide a temporary fistula for cerebrospinal fluid egress through the orbit; this procedure is used in other non-infectious processes associated with elevated ICP with optic nerve compression.

Corticosteroids have been used successfully in anecdotal reports. One study reported lower rates of visual deterioration and blindness in patients receiving hydrocortisone (100–250 mg/day) for prevention of amphotericin B side effects.<sup>156</sup>

Seaton et al postulated that the host inflammatory response contributed to optic nerve compression and that the corticosteroids ameliorated this response.<sup>156</sup>

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# Geographic, travel, and occupational fungal infections

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For the purposes of epidemiology, fungal infections are considered to have been caused by one of two types of fungi: opportunistic or endemic. The opportunistic fungi include *Aspergillus*, *Candida*, *Fusarium*, and *Rhizopus* species; some of the fungi more traditionally characterized as endemic fungi, including *Histoplasma*, *Blastomyces*, and *Coccidioides*, may also present as an opportunistic infection in the immunocompromised patient. However, the organisms considered to be opportunistic fungi do not cause endemic or geographically localized diseases. *Aspergillus*, *Candida*, *Fusarium*, *Rhizopus* species and the like are ubiquitous, being found throughout the world. The epidemiology, risk factors, and pathogenesis for these organisms are discussed in other chapters in this textbook.

The endemic fungal infections, whose clinical manifestations, pathogenesis, and treatment are discussed in detail in other chapters, tend to fall into geographic patterns. Most persons diagnosed with an infection due to any of these fungi are likely to live in fairly discrete portions of the world with specific ecologic and climatic conditions. However, with the ease of national and international travel, patients may present with a fungus infection contracted in a remote location of the world; questioning the patient regarding travel may be the most important part of the diagnostic evaluation. Likewise, certain occupational or recreational activities might put a person at risk for these endemic fungi. Many persons infected with these fungi have adequate host defenses. Therefore many of these infections will cause few or no symptoms at the time of infection but later reactivate to systemic disease if the human host becomes immunocompromised.

The purpose of this chapter is to give a brief summary of the geographic niches for the endemic mycoses and to review some of the historical and clinical aspects of these fungal infections.

## Histoplasmosis

*Histoplasma capsulatum* is the cause of the endemic mycosis histoplasmosis and was first discovered to be a cause of disease in humans by Darling in 1906. He described an autopsy on first one and subsequently two more individuals during his

work in Panama.<sup>1</sup> This is of interest because further cases were not described in Panama for decades. The next case of histoplasmosis was described in Minnesota,<sup>2</sup> which like Panama is not in the highest endemic area. At that point, however, the history of histoplasmosis shifted to the center of the United States, where histoplasmosis is now recognized to be common. As described in an articulate and entertaining report by Sell,<sup>3</sup> Nashville, Tennessee, became the focus for investigation of this fungus. In 1934 the blood smear from an infant was found to have organisms similar to those described in Darling's original case. Cultures of specimens of bone marrow and blood from an autopsy performed soon after the patient's death revealed a fungus, *Histoplasma capsulatum*.

Over the next decade an additional 70 or so cases of histoplasmosis were summarized in a review by Meleney; all were of the disseminated form and were fatal.<sup>4</sup> A filtrate of the mycelial form of a fungus was used as a skin test to identify subclinical or asymptomatic cases of infection. This led to a hallmark article by Christie and Peterson in 1945, which changed the understanding of interactions of fungi with human hosts.<sup>5</sup> Children with pulmonary calcifications that had been thought to be due to tuberculosis were skin tested with histoplasmin and tuberculin antigens; some children had a positive reaction to both antigens, whereas 49% were histoplasmin positive and tuberculin negative, and only 33% were histoplasmin negative and tuberculin positive. This indicated that most healthy children with pulmonary calcifications did not have tuberculosis but had mild or asymptomatic histoplasmosis.<sup>5</sup> Studies by Palmer<sup>6</sup> and Edwards et al<sup>7</sup> of military recruits and others subsequently confirmed that a large number of healthy persons may be infected with *H. capsulatum* early in life, with resultant pulmonary calcifications but little or no clinical illness. These studies found that most cases of histoplasmosis occur in the central section of the United States. However, there have been reports of cases throughout the eastern half of the United States and Latin America.<sup>8</sup> Infections have also been reported, albeit less commonly, in Europe and in Asia, including Malaysia, Thailand, India, and Indonesia.<sup>8,9</sup>

Duncan described a different form of histoplasmosis in Africa in 1958.<sup>10</sup> The organisms appear the same in the mycelial form, but the yeast form is considerably larger. This strain, known as the *H. duboisii* form or large-form African



histoplasmosis, is considered to be a variety of *H. capsulatum*; a similar pattern of illness is seen in this African histoplasmosis form.<sup>9,10</sup>

In the United States it was estimated that there were 200,000 new cases of histoplasmosis per year in 1968.<sup>11</sup> This accounts for the 80–95% positive skin test rates for children in some highly endemic areas. In the presence of a depressed immune system, as occurs with HIV infection, corticosteroid therapy or organ transplantation, progressive disseminated histoplasmosis may occur.<sup>8,9,12</sup> In some of the endemic areas of the country, histoplasmosis is the opportunistic infection that most frequently leads to a diagnosis of AIDS.

Careful attention should be paid to occupational or recreational exposure to bird droppings or bat guano, since either can act as a growth nutrient for *H. capsulatum*.<sup>8,12</sup> The best, and perhaps the only, way to make a diagnosis of acute symptomatic pulmonary histoplasmosis, which is manifest with fever, chills, myalgia, dyspnea, and hypoxia, is to obtain a history of exposure.<sup>12</sup> This would include activities such as cutting down trees that had been known to be bird roosts, destroying chicken coops that had remained unused for a long time, or spelunking in caves known to have large bat populations.

## Blastomycosis

The first reported case of blastomycosis was by Gilchrist in 1894 with subsequent isolation of the cause: a fungus, *Blastomyces dermatitidis*.<sup>13</sup> This dimorphic fungus has a characteristic geographic niche also. The area in which it is endemic is similar to that for histoplasmosis but perhaps a bit more restricted and includes states surrounding the Mississippi and Ohio Rivers in the United States.<sup>14</sup> Most cases have been described in Arkansas, Kentucky, Mississippi, North Carolina, Tennessee, Louisiana, Illinois, Minnesota, and Wisconsin.<sup>15</sup> Most are isolated infections but a few epidemics of infection from point sources have also been described; epidemic cases are thought to have been related to a common source outbreak.<sup>16</sup> The epidemiology of blastomycosis is not as well understood as that of histoplasmosis, primarily because of the lack of a sensitive and reliable skin test or any other in vitro marker of prior infection. From 1896 to 1968, 1476 cases were reported.<sup>17</sup> Prevalence rates as high as from 0.5 to 4 cases per 100,000 population per year were reported. Cases have also been reported in Canada in the provinces of Manitoba, Ontario, Alberta, and Saskatchewan.<sup>15</sup>

The outbreak of blastomycosis in Eagle River, Wisconsin, in 1985 was the first time that the organism was isolated from soil in conjunction with an epidemic.<sup>18</sup> A second isolation of *B. dermatitidis* was reported from a nearby location in Wisconsin.<sup>19</sup> In both cases the specimens were of wet earth containing a high organic content from animal droppings and thereby gave proof of the organism's existence in microfoci in soil.<sup>20,21</sup>

Blastomycosis has been described in Africa and India, as well as in Israel, Lebanon, Saudi Arabia, and Mexico. These cases are rare. There had been previous reports of blastomycosis in South America and Central America; most likely these cases were of paracoccidioidomycosis, which was once known as South American blastomycosis. This terminology has been abandoned, because it is preferable to use the term that identifies

the infecting fungus, *Paracoccidioides brasiliensis*, and because the infection from *B. dermatitidis* has been documented to occur outside North America.<sup>16</sup>

The occupational or recreational exposures of importance in blastomycosis are those that lead to contact with the soil.<sup>15</sup> Specifically this includes fishing, hunting, farming, construction work or other activities that involve disturbances of moist earth.<sup>19</sup> In several of the epidemics of infection, soil near bodies of water was thought to be responsible. Whether water is the primary transmission factor or simply poses a greater potential risk because of the recreational opportunities around waterways remains to be determined.<sup>21</sup>

Blastomycosis typically is described in normal human hosts. The infection can either remain localized in the lung or disseminate to skin, bone, genitourinary tract or other organs. Immunodeficiency of the host will increase the likelihood of disseminated disease, including involvement of the central nervous system, although most cases of disseminated infection are not in patients with documented problems with the immune system. Blastomycosis in conjunction with HIV infection has not been commonly described; less than 50 cases have been documented in patients with AIDS.<sup>22,23</sup> When this does occur, widespread dissemination has been the rule.

## Cryptococcosis

As reported by Kwon-Chung and Bennett, Busse and Buschke independently described, in 1894 and 1896 respectively, the recovery of an organism from a 31-year-old woman with a sarcoma-like lesion of her tibia.<sup>24</sup> An encapsulated yeast was isolated from peach juice in that same era, which later was shown to be the same organism, *Cryptococcus neoformans*.<sup>24</sup> Although the infection has also been known as torulosis and European blastomycosis, cryptococcosis is the appropriate name. There are two varieties of this species: *Cryptococcus neoformans* var. *neoformans* and *Cryptococcus neoformans* var. *gatti*. The var. *gatti* has been prevalent only in tropical and subtropical regions. However, the illnesses due to the two strains are similar.

Unlike histoplasmosis and blastomycosis, cryptococcosis is not limited to geographic regions but is worldwide.<sup>25</sup> The infection is obtained by pulmonary inhalation and most persons with this infection remain asymptomatic. However, with immunosuppression, this fungus may cause systemic and life-threatening infection, particularly meningitis.<sup>25</sup> There was a marked increase in the number of cases of cryptococcal meningitis in the 1980s as AIDS was identified.<sup>26</sup> At one point, cryptococcosis was the fourth most commonly diagnosed opportunistic infection in patients with HIV infection. Unlike many of the endemic mycoses, cryptococcosis is common throughout the world in AIDS patients.<sup>26</sup>

There are no particular occupational risk factors for cryptococcal infection, although bird fanciers and pigeon breeders may have a recreational basis for increased exposure. Most persons with such a history have not had clinical disease but only antibody evidence of prior infection. For disease due to cryptococcosis, mechanisms of immunosuppression by steroid use, lymphoma or sarcoidosis are considered major factors outside HIV infection.<sup>25</sup>

## Coccidioidomycosis

Like histoplasmosis, coccidioidomycosis was described first in Latin America in the southern hemisphere. In 1891 a 21-year-old medical student named Alejandro Posadas, working in the pathology laboratory of Robert Wernicke in Buenos Aires, diagnosed a patient with an unusual skin tumor. In 1892 the patient's illness was described in Argentina,<sup>27</sup> with Wernicke reporting the same case in Germany.<sup>28</sup> Four years later, Rixford and Gilchrist reported the first North American case from a Portuguese immigrant patient in California.<sup>29,30</sup> There is a rich history of the mycology and ecology of this fungus in the first three decades of the 20th century.<sup>31</sup>

Coccidioidomycosis and histoplasmosis have similar disease patterns, and probably the numbers of cases of each diagnosed annually in the United States are similar also.<sup>31</sup> Most patients have minimal to mild disease, with progressive disease developing in only 1% of infected persons.<sup>31,32</sup> There is a clearly recognized racial and ethnic group predisposition to disseminated infection. African-Americans and persons of Filipino or other Asian descent have a much greater risk of having disseminated coccidioidomycosis.<sup>31,33</sup> In addition, immunosuppression of any type, including the mild form of immunosuppression associated with pregnancy, will lead to an increased risk of dissemination.<sup>31,33</sup>

Coccidioidomycosis occurs in the Lower Sonoran Life Zone.<sup>31</sup> This corresponds to central California, Arizona, Nevada, Utah, Texas, New Mexico, and northern Mexico. In Central America, Guatemala and Honduras have endemic foci for this fungus. In South America, cases are diagnosed in Argentina, Paraguay, Bolivia, Venezuela, Uruguay, and Ecuador.<sup>33</sup> These areas have alkaline soil, hot summers, mild winters, and not much rainfall.

There is a recreational or occupational risk for coccidioidomycosis. Exposure to dust, dirt or disturbed soil in the endemic area raises the potential for infection. Construction and archaeological excavation workers or military personnel on maneuvers are at increased risk for infection.<sup>34</sup> An earthquake in the late 1990s in Los Angeles led to a substantial increase in the number of cases.<sup>35</sup>

As with histoplasmosis, skin tests positive for fungal antigens of coccidioidomycosis are frequently found in children and adults. In the San Joaquin Valley, prevalence rates of 50–70% have been documented. Lifetime exposure in these areas is not required for infection, however. There are reports of infection far outside the endemic area in persons exposed to dust from the coccidioidomycosis region.<sup>32</sup> In addition, persons may have had only a brief visit to the area before returning home with their incubating infection.<sup>34</sup>

## Sporotrichosis

*Sporothrix schenckii* is the cause of sporotrichosis, which usually is a chronic infection of the skin and subcutaneous tissue. Like blastomycosis, infection with this fungus was first diagnosed at the Johns Hopkins hospital in Baltimore in the late 1890s.<sup>36</sup> Unlike blastomycosis, this infection does not typically begin with pulmonary inhalation but rather with cutaneous inoculation.<sup>12,37</sup>

Sporotrichosis is global in distribution but is found primarily in fairly temperate zones of North America, South America, and Japan.<sup>37</sup> There are regions with particularly high frequencies of infection, including areas in Peru, Brazil, Mexico, France, and other areas. Pappas and colleagues described 238 cases over 3 years from a hyperendemic area in the south central highlands of Peru.<sup>38</sup>

Although there are no particular geographic risk factors, there are occupational risk factors for sporotrichosis. The organism grows in soil, particularly soil mixed with hay or moss or with high amounts of organic matter, and is inoculated after accidental puncture of the skin. Therefore, gardening, farming, floral work or other activities that involve exposure to hay or moss, and particularly roses, have been associated with this organism.<sup>37</sup> Two reports of outbreaks of sporotrichosis were of particular interest. Hajjeh and colleagues<sup>39</sup> described workers with topiary trees in an amusement park in Florida who developed the infection. Dooley and colleagues<sup>40</sup> reported an outbreak traced to hay bales used in a Halloween spook house. Workers who set up the hay bales for supports were involved; however, one youngster who had been forbidden by his parents to enter the exhibit was proven to have been disobedient by the diagnosis of sporotrichosis.<sup>40</sup>

In addition, contact with animals that may have the fungus on their skin may transmit infection. This has been demonstrated from cat bites or scratches or simply from nuzzling the cat;<sup>41</sup> veterinarians are the most likely to be infected by this route. Epidemics of infection in South African gold mines due to contaminated wood supports have been described.<sup>42</sup>

With cutaneous sporotrichosis, children and young adults are the typical subjects. Excessive alcohol use has been associated with progressive infection as has any condition that leads to immunosuppression, such as HIV infection.<sup>37</sup> Pulmonary sporotrichosis with cavitary lesions may be found in patients with chronic obstructive pulmonary disease.<sup>37</sup>

## Paracoccidioidomycosis

Paracoccidioidomycosis is due to the fungus *Paracoccidioides brasiliensis*; it is the only mycotic disease geographically restricted to Latin America.<sup>43</sup> Humans were the only known hosts susceptible to natural infection until cases were diagnosed in armadillos.<sup>44</sup> The pattern of infection is similar to that of histoplasmosis, coccidioidomycosis and, to a lesser degree, blastomycosis in that primary infection is thought to be pulmonary and is most commonly asymptomatic. Later in life, as the immune system has some perturbation, the previously asymptomatic infection can develop into systemic disease. In paracoccidioidomycosis, this usually occurs in middle age in older adult men who present with pneumonia, mucocutaneous lesions or skin lesions.<sup>45</sup>

Paracoccidioidomycosis has been diagnosed in patients in the United States, Canada, Europe, and Asia. However, each of these couple of dozen cases has arisen in people who lived in Latin America at some point before the diagnosis.<sup>43</sup> The endemic area for paracoccidioidomycosis ranges from Mexico to Argentina, with the largest number of cases being reported from Brazil, followed by Venezuela, Colombia, and Ecuador.<sup>43</sup> As with histoplasmosis and blastomycosis, there are probably hyperendemic subregions within these countries. In other words,

the infection remains relatively rare even in these endemic areas, and the explanation is not clear as to why the disease develops from the infection in one person and not another.

There are gender differences in this infection. Systemic disease is unlikely to develop in children and young adults, as in blastomycosis.<sup>16</sup> Skin tests with an antigen of *P. brasiliensis* (paracoccidioidin) have positive results of approximately 60–70% in healthy children and adults of both sexes.<sup>43</sup> However, clinical disease is found almost exclusively in men. This may be due to a hormonal effect on fungal growth, but the reason is not fully understood.<sup>43</sup>

Severe and progressive disease is known as subacute infection or juvenile form. The progressive adult form is more likely to be seen in older men with chronic disease.<sup>45</sup> This is the only fungal infection that responds to sulfonamide therapy, although azole or amphotericin therapy is more reliable.<sup>43</sup>

Because the infection may remain dormant with later activation and subsequent disease up to three or four decades after primary infection, the major way to make the diagnosis outside Latin America is a history of travel or previous residence in Central or South America.<sup>43,45</sup> Diagnosis is confirmed by observations of the characteristic numerous buds with the refractile cell wall of the fungal elements on KOH examination of sputum or pus, by culture or by histologic examination of tissue.

## Penicilliosis

The only dimorphic fungus of the genus *Penicillium* is *Penicillium marneffei*. This fungus has been described as a cause of systemic illness in HIV-infected residents of south east Asia or southern China.<sup>46</sup> As described by Duong,<sup>46</sup> the organism was first isolated in 1956 from bamboo rats in Vietnam.<sup>47</sup> The first case in a human was described in 1959 after the author had accidentally inoculated the organism into his finger; he treated himself successfully with oral nystatin.<sup>47</sup> This agent has not been associated with cure subsequently. The next case was described by DiSalvo et al.<sup>48</sup> in a minister who had worked in Vietnam and later developed Hodgkin's disease requiring a splenectomy. The spleen grew *P. marneffei*. Before the surge in the HIV epidemic in south east Asia, penicilliosis was uncommon. However, since 1988 the infection has been diagnosed much more frequently; Supparatpinyo and colleagues report that 15–20% of all AIDS-related illnesses are due to this fungal infection.<sup>49</sup> It is the third most common opportunistic infection in this patient group in Thailand, following tuberculosis and cryptococcosis.<sup>50</sup>

The organism grows as a mould at 25°C and as a yeast at 37°C. Unlike *Histoplasma* or *Blastomyces*, which divide by budding, *P. marneffei* divides by fission. The histology is similar to that seen in histoplasmosis or blastomycosis in that both suppuration and granulomatous changes in the tissue may be found in response to the fungus.

Although the organism has been associated with bamboo rats in south east Asia, there is no direct correlation of particular cases between the humans and animals.<sup>51</sup> On occasion, the fungus has been isolated from the soil in areas where the rats live.<sup>52</sup> This may be similar to the association of blastomycosis with beavers;<sup>20,21</sup> the animal may contribute to the organic component of the soil that promotes the growth of the fungus rather than being the reservoir of infection.

Most cases of penicilliosis in Thailand have been in men, and the vast majority of them are immunosuppressed by HIV infection.<sup>44,48</sup> In contrast, cases in southern China were reported in people with normal immune systems.<sup>44</sup> A handful of cases have arisen in people from the United States or Europe, but all had exposure to south east Asia or China.

The manifestations of *P. marneffei* have been systemic illness with skin lesions, cough, lymphadenopathy, and weight loss.<sup>46,50</sup> Manifestations of the disease are considered similar to those of histoplasmosis in HIV-infected individuals. Amphotericin has been associated with improvement, but relapse is common once the antibiotic is stopped; itraconazole as maintenance therapy has been successful in preventing relapse.<sup>53</sup>

As with paracoccidioidomycosis, it is unlikely that a diagnosis of *P. marneffei* will be made in the Western world unless a careful history of exposure is obtained. Culture or histology would identify the organism once the diagnosis has been considered.

## Summary

A number of systemic fungal infections have specific characteristic geographic niches. A careful history may be the only means to make the diagnosis. Many of these endemic fungi will cause asymptomatic primary infection in a portion of the population that lives in the endemic area. Either at the time of primary infection or at a much later time, the disease may progress with lymphohematogenous dissemination to various organs. Skin, nodes, bone marrow, lungs, and the central nervous system are the most common sites of progressive infection. Culture and histologic examination of tissue will confirm the diagnosis of fungal infection, and treatment with either amphotericin or an azole, such as itraconazole or fluconazole, may well cure the infection.

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# Mycotoxins and their effects on humans

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The term “mycotoxin” describes a chemically diverse group of low molecular weight organic substances produced primarily by moulds, although some yeasts and basidiomycetes (mushrooms) have the capacity to form mycotoxins. Excluding compounds produced by mushrooms, moulds are known to produce about 300–400 compounds that are recognized as mycotoxins.<sup>1</sup>

Mycotoxins form in hyphae where they may remain, be incorporated into conidia during conidiogenesis, or be expelled into the environment. These substances are thought to be produced by fungi as ecologic survival aids designed to reduce competition for nutrients and living space by other fungi and organisms such as bacteria, insects, and arachnids. Depending on the mycotoxin, various effects on protein, DNA, RNA synthesis or disruption of cell membranes are produced, resulting in either impaired cellular function or death.<sup>2</sup>

Given the adverse effect of these compounds upon cellular metabolism, the saprophytic to parasitic nature of fungi, and their frequency in the environment, it is no surprise that both humans and animals are exposed to mycotoxins that may result in harmful effects. This has been the case throughout recorded history, with mycotoxins even being implicated in one of the Ten Plagues of Egypt.<sup>3</sup>

There are three primary manners in which humans and animals may be exposed to mycotoxins; eating contaminated food like cereals and grains, by skin contact and subsequent absorption of the mycotoxins, or by inhalation of mycotoxins or fungal elements containing mycotoxin. Of the three, the first is the most common and may result from pre-harvest growth of mycotoxin-producing fungi on the grain, fungal growth during storage, or by contamination of surfaces under conditions favorable for fungal growth. Mycotoxin contamination of grain can be a significant problem in underdeveloped countries where reliance on a single grain food source with heavy intake of the cereal is common.<sup>4</sup> In these environments, *Aspergillus* spp., which produce aflatoxins and ochratoxins, *Penicillium* spp., which produce ochratoxins, and *Fusarium* spp., which produce trichothecenes and fumosins, are the most relevant species.<sup>4</sup>

A few mycotoxins can be absorbed through the skin or mucous membranes, and may induce necrosis in addition to systemic effects on other rapidly dividing tissues such as

the gastrointestinal tract and the hematopoietic system. At present, some trichothecenes are known to possess this property. These mycotoxins have relevance with respect to the production and use of biologic weapons and are thought to contribute to indoor health problems following water damage in buildings.

Absorption of mycotoxins through the respiratory system, by inhalation of mycotoxin-containing fungal elements, conidia or hyphal fragments, is the route of entry into the human body which has recently become of significant interest. Environments where this can occur include not only industrial processes that involve fungi or commodities contaminated with fungi, such as milling, but also indoor living or working settings with a high amount of mould growth. This latter situation, which has been associated with a somewhat variable and as yet not well-defined complex of signs and symptoms in affected individuals (“toxic mould syndrome”), has not only generated a high level of interest in mycotoxins. It has also illustrated a primary problem related to the association of mycotoxins with medical syndromes or diseases: that is, it has been extremely difficult to definitively link a syndrome or disease with a mycotoxicosis. As has been pointed out by a number of authors, the simple presence of a species of mould does not necessarily imply the presence of mycotoxin.<sup>1,2</sup> The conditions necessary for production of mycotoxin by a given species may be different from those necessary for growth of the mould.<sup>2</sup> In addition, not all strains of a given species are capable of mycotoxin production and, of those that do produce the substances, there can be significant variability in efficiency of production.<sup>1</sup> Further, the effects of mycotoxins are influenced by a large number of factors, including not only the specific mechanism of action of the specific mycotoxin but also the amount and duration of exposure, the general health, age, and sex of the subject, and a host of synergistic effects related to genetics, diet, and interaction with other potential pathogenetic facilitators such as alcohol, infectious agents, and deficits in caloric or vitamin intake.<sup>1</sup>

These difficulties have been particularly vexing with respect to clarifying the association of mycotoxins with chronic conditions that require a long time to develop. Acute intoxications have been less problematic in some aspects, although by no means has the association been clear in all

cases. Fortunately, as advancements in both epidemiology and molecular medicine have occurred at a rapid rate over the last several decades, some of these associations have been clarified. Some syndromes and diseases have been clearly established as related to mycotoxins, others that were initially thought to have an association have been demonstrated to either not be linked or have a tenuous or weak association at best, while others require more study before any conclusion can be reached.

## Aflatoxins

Aflatoxins are difuranocoumarin derivatives, of which there are over a dozen, with types B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub> being the major types, and B<sub>1</sub> being the main aflatoxin produced. Aflatoxins are produced by *Aspergillus* spp., primarily *A. flavus* and *A. parasiticus*, but a few other species in the genus may also produce these substances.<sup>1</sup> The commodities affected include corn, figs, cottonseed, peanuts, certain tree nuts, and tobacco.

The primary target organ is the liver, where cytochrome P450 enzymes convert aflatoxins to a reactive form, which can bind to both proteins and DNA.<sup>1</sup> Detoxification involves conjugation of this reactive form by a glutathione S-transferase, with subsequent excretion of the conjugate. There appears to be a significant difference in susceptibility to aflatoxins amongst different animal species, and it is thought that differences in both cytochrome P450 and glutathione S-transferase systems underlie these differences.<sup>1</sup>

## Acute aflatoxicosis

Acute toxicosis from aflatoxins has been associated with contaminated grains, particularly maize, and has manifest as an acute hepatitis with centrilobular necrosis and steatosis by histopathologic examination.<sup>5-8</sup> Mortality was up to 25% in a large series of cases in India. The lethal dose for acute toxicosis for aflatoxin has been approximated to be 10–20mg and in this series, some patients were estimated to have ingested up to 6 mg in a single day.<sup>1,6,7</sup>

Kwashiorkor is a childhood disease that has manifestations of severe protein deficiency, hepatic steatosis, and ascites. The disease has been associated geographically with the seasonal occurrence of aflatoxins in food.<sup>9</sup> Animals given dietary aflatoxin demonstrate some of the conditions associated with kwashiorkor, including hypoalbuminemia, hepatic steatosis, and immunosuppression. Many of the manifestations of kwashiorkor in children, including the hepatic steatosis due to decreased apoproteins, the decrease in albumin and other proteins with ascites, and the decrease in antioxidants including glutathione, are consistent with the hepatotoxic effects of aflatoxins. Additionally, aflatoxins have been detected in the livers of children who died with the disease.<sup>10</sup> Despite a fair amount of circumstantial evidence, the case firmly establishing aflatoxins as the perpetrators of kwashiorkor is yet to be made and awaits further studies.

Another disorder associated with hepatic steatosis is Reye's syndrome, an often fatal acute encephalopathy that sometimes occurs after a viral infection, primarily in children or adolescents. The disorder was initially proposed to have a possible association with aflatoxins because of the finding of aflatoxins

in some Reye's syndrome patients; however, subsequent studies have not supported this proposal.<sup>11-13</sup>

## Chronic aflatoxicosis

A good example of how careful epidemiologic studies and improvements in molecular techniques have come together in recent years to clarify the relationship of a mycotoxin to a specific disease is the relationship between aflatoxin B<sub>1</sub> and hepatocellular carcinoma (HCC). While initial studies presented somewhat conflicting results, it has now been accepted by most that aflatoxin B<sub>1</sub> is involved in the pathogenesis of some cases of HCC, and it has been classified as a Group 1 carcinogen by the International Agency for Research on Cancer.<sup>1</sup>

Worldwide, the major risk factor for development of this malignancy is the hepatitis viruses B (HBV) and C (HCV), with an estimated 50% of cases of HCC associated with HBV and 25% of cases associated with HCV.<sup>14</sup> In several major epidemiologic cohort studies, using the presence of an aflatoxin B<sub>1</sub>-DNA adduct whose excision product (aflatoxin B<sub>1</sub>-guanine) can be detected in urine,<sup>15</sup> it was determined that aflatoxin B<sub>1</sub> exposure is associated with an increased risk of developing HCC. However, it was only when investigators began to examine the possible relationship between HBV infection and aflatoxin exposure that the true potential impact of this mycotoxin as a co-carcinogen became clear. In a large cohort study from China, aflatoxin exposure revealed a relative risk of 3.4 for development of HCC, the presence of HBV revealed a relative risk of 7.3, and, most importantly, the relative risk for those patients who were positive for both aflatoxin exposure and HBV infection was 59.<sup>16</sup> Additionally, it has been demonstrated that a very specific mutation in the *p53* tumor suppressor gene (G→T transversion of the third base of codon 249), a gene found mutated in many human cancers, has been found frequently in association with HCC in areas of high aflatoxin exposure and very rarely in tumors from patients who reside in areas with little aflatoxin exposure.<sup>16</sup> Both epidemiologic and molecular evidence points to the mycotoxin aflatoxin B<sub>1</sub> as an important factor in the development of liver cancer in patients with HBV.

## Ergot alkaloids

These substances are indole alkaloids and there are different classes that have slightly different structures and different relative actions, with their effects including smooth muscle contraction, central sympatholytic activity, and peripheral  $\alpha$ -adrenergic blockade. Ergotamine tartrate (an amino acid alkaloid) is the most potent vasoconstrictor and also causes contraction of uterine smooth muscle, although not when given orally.<sup>17</sup> Ergonovine and methylergonovine (amine alkaloids) are very effective at causing uterine contraction (oxytocic effect) while having minimal vasoconstrictive effect without any sympatholytic or  $\alpha$ -adrenergic blocking effect.<sup>17</sup> The group with the most sympatholytic and  $\alpha$ -adrenergic blocking effect is the dehydrogenated amino acid alkaloids, dihydroergotamine and dihydroergotamine.<sup>17</sup>

Ergot is produced in sclerotia of the genus *Claviceps*, which are pathogens of grasses and grains, and consists of a mixture of alkaloids, in addition to some other compounds.

Ingestion of grains or grain products contaminated with ergot results in ergotism, a mycotoxin poisoning which has been postulated to have been responsible for large outbreaks from antiquity through the 19th century, and is thought by historians to be responsible for hundreds of thousands of deaths.<sup>18,19</sup> Although large epidemic outbreaks of ergotism are no longer seen, prevented by modern agricultural techniques, because of the medicinal use of ergotamine in the treatment of migraine headaches, iatrogenic ergotism still occurs.

## Ergotism

The vasospasm induced by ergot can affect any organ, resulting in ischemia to the tissue and manifestations related to anoxia and infarction of the affected organ. There are two major clinical syndromes associated with ergot poisoning, the signs and symptoms of which have been gleaned from descriptions of affected individuals during the large outbreaks. The two types are the gangrenous and convulsive syndromes. While some symptoms and signs, such as lassitude, nausea and vomiting, diarrhea, back pain, cutaneous ischemic changes in hands and feet, and mental impairment, could be common to both syndromes, gangrene did not usually occur in the patient with the convulsive syndrome and convulsions did not usually occur with the gangrenous syndrome.<sup>17</sup>

The gangrenous syndrome often began with several weeks of intense burning pain (St Anthony's fire), usually in the extremities, the sites most often affected.<sup>17</sup> As the patient continued to consume ergot, ischemia of the affected extremities lead to gangrene, most often dry gangrene, and subsequent auto-amputation of the limb or limbs, as the effect was usually symmetric. As the vasculature of any organ could be involved, intestinal infarction, renal failure, angina pectoris or acute myocardial infarction, or blindness from ischemic infarction of the respective organ could also occur.

The feeling of insects crawling on the skin (fomication) was a very common symptom associated with the convulsive form early in the course, and with increasing toxicity the poisoned individual began to suffer pain, colonic contractions of the digits and extremities, and weakness.<sup>17</sup> Disorientation, dementia, and sensory disturbances could also occur. Ischemia of the central nervous system sometimes lead to hemiparesis, paraplegia or pseudotabes dorsalis. With very severe poisoning, repetitive seizures culminated in death.

It is thought that the different forms of ergotism may have resulted from differing combinations and quantities of ergot alkaloids that are produced by the different species of *Claviceps*, although this has never been proven, nor will it likely ever be since outbreaks of naturally occurring ergotism are extremely rare in modern times. Possibly lending support to this theory, however, is the fact that the type of ergotism seen today, medical ergotism due to the overdose of the drug ergotamine used to treat migraine headaches, has demonstrated only a single clinical syndrome, that of arterial vasospasm with resultant ischemia. The convulsive syndrome has not been reported in this setting. Cases of medical ergotism are sometimes seen even when the patient is on a low dose of ergotamine when the patient is also being treated with drugs that can inhibit the cytochrome P450 enzymes, as has been reported in HIV-infected patients on the antiretroviral zidovudine or in patients taking the antibiotic macrolides.<sup>20,21</sup>

## Ochratoxin

Ochratoxin A is a dihydroisocoumarin produced by *Aspergillus ochraceus* and at least seven other *Aspergillus* species, including *A. niger* (used in enzyme production for human consumption) and *A. carbonarius* (found on wine grapes).<sup>22</sup> *Penicillium verrucosum*, found on some grains and corn, also produces this mycotoxin. Ochratoxin A has been detected in a host of substances including a variety of grains such as barley, oats, rye, and wheat, and other commodities such as coffee beans, cocoa, various nuts, spices, and wines and beer.<sup>1,22</sup> It is fat soluble and poorly excreted, and as such, finds its way into food animals (particularly pork) through feeds, from which it can be consumed by humans.<sup>19</sup> This substance has a number of biochemical effects, including inhibition of phenylalanine metabolism, inhibition of mitochondrial ATP production, and the stimulation of lipid peroxidation.<sup>1</sup>

## Ochratoxicosis

The literature does not contain case reports of acute toxicity caused by ochratoxin in humans. However, it has been shown to be a potent nephrotoxin in all animal species tested, and is felt to be responsible for porcine nephropathy, a significant problem for pork-producing countries.<sup>1</sup> Additionally, animal studies have shown that ochratoxin is hepatotoxic, an immunosuppressant, a teratogen, and a carcinogen.<sup>1</sup>

Ochratoxin is, however, also a good example of how difficult it is to establish mycotoxins as definite etiologic agents in human medical conditions that require a long period to develop. It has been postulated that ochratoxin exposure results in a progressive chronic nephritis in some areas of eastern Europe (Balkan nephropathy) and in tumors of the urinary tract in humans. Some studies have demonstrated increased levels of ochratoxin in foods and in serum in the homes and blood, respectively, of patients with these disorders relative to patients in the same area without these disorders.<sup>1,22</sup>

Studies examining links between mycotoxins and chronic disorders face a variety of difficulties. For example, there is great variation in mycotoxin amounts from crop to crop or commodity to commodity and it is difficult to estimate long-term consumption. There is minimal information on how food preparation methods such as cooking affect mycotoxins. It is known that there is genetic variation amongst individuals in their detoxification abilities (genetic variation in cytochrome P450 enzymes, for example) and because often the methods of assessing exposure are internal methods that are more applicable to estimating individual exposure rather than a population, very large studies are necessary to permit any degree of generalization, and these can be very expensive. As summarized well by Clark and Snedeker, assessing the link between ochratoxin and Balkan nephropathy and urogenital tract tumors has faced these problems and more, and at this time an unequivocal link is not possible.<sup>22</sup> Despite this, there is very suggestive evidence, based on the studies that have been done, that the nephrotoxic and carcinogenic properties of ochratoxin are applicable to humans. Ochratoxin may act as a co-carcinogen similar to aflatoxin. The International Agency for Research on Cancer has labeled ochratoxin A as a possible carcinogen (category 2B), and many nations have established maximum allowable levels in foods and commodities.<sup>1,22</sup>

## Fumonisin

This group of mycotoxins is produced by members of the genus *Fusarium*, with *F. verticillioides* (formerly *F. moniliforme*) probably the most important, but other species such as *F. proliferatum* may also produce fumonisins, as may *Alternaria alternata*.<sup>1</sup> *F. verticillioides* is present on nearly all corn and may cause “seedling blight,” “ear rot” or “stalk rot” but also may be present without noticeable adverse effect on the corn. Fumonisin production is strain dependent in all species that potentially produce them.

Biochemically, these mycotoxins consist of a 20 carbon aliphatic backbone with two ester-linked hydrophilic side-chains, and the compounds bear a resemblance to sphingosine.<sup>19</sup> This resemblance appears to play a role in inhibiting sphingolipid metabolism in animals. Fumonisin B1 inhibits N-acyltransferase (ceramide synthase), which is involved in the conversion of sphinganine and sphingosine to ceramide in sphingolipid biosynthesis.<sup>23</sup> Since sphingolipids are major components of cell membranes, their effects are wide-ranging and they are known to cause liver cancer in rats, pulmonary edema in pigs and are the cause of the naturally occurring and severe, fatal leukoencephalomalacia in horses.<sup>24-26</sup>

In humans, fumonisins have been noted to have a possible link with esophageal cancer, based on the co-occurrence of a high incidence of *F. verticillioides* contamination of grain and a high incidence of esophageal cancer in areas of South Africa, China, and Italy.<sup>1,19</sup> *F. verticillioides*-contaminated cornmeal has been demonstrated to produce premalignant changes in the esophagus and stomachs of rats and mice, in addition to liver carcinoma.<sup>27</sup> Further case-control epidemiologic studies are necessary to establish the link between fumonisins and esophageal carcinoma in humans.

Evidence that fumonisins may induce neural tube defects in humans arose out of a high incidence of anencephaly and other neural tube defects along the Texas–Mexico border in the early 1990s, associated with an epidemic of equine leukoencephalomalacia that had occurred in Texas. To contain the leukoencephalomalacia outbreak, animals were not allowed to eat corn, as it was contaminated with *F. verticillioides*. Humans, however, continued to consume the corn from this harvest. Careful epidemiologic work by Missmer and colleagues, who conducted a case-control study correlating fumonisin levels in corn tortillas, consumption amounts of tortillas during pregnancy, and a surrogate marker for fumonisin in mother's postpartum serum (sphinganine:sphingosine ratio), suggested that fumonisin exposure in pregnancy played a role in the development of neural tube defects.<sup>28</sup> As these authors noted, other studies had demonstrated that inhibition of sphingolipid biosynthesis adversely affects uptake and binding of folate, and that an animal model exposed to fumonisins developed neural tube defects, the effect of which was prevented by folate administration, lending further support to the hypothesis related to the teratogenic potential of fumonisins.<sup>28</sup>

## Gliotoxin

This mycotoxin is an epipolythiodioxopiperazine, a group of mycotoxins that possess a disulfide bridge that appears to be involved in the toxic effects.<sup>29</sup> Gliotoxin is produced by

*A. fumigatus* (and no other members of the genus), *Trichoderma virens*, *Penicillium* spp., and *Candida albicans*. The mechanisms by which gliotoxin induces toxicity are thought to be by conjugating with thiol residues on proteins and by generating reactive oxygen species through oxidation of the reduced dithiol to the disulfide form.<sup>29</sup> Gliotoxin has been demonstrated to have a number of immunosuppressive actions, including the ability to inhibit oxidative killing by neutrophils and macrophages, the phagocytic ability of macrophages, antigen-mediated activation of lymphocytes and cytotoxic T cell activation, the ability of T-helper lymphocytes to secrete  $\gamma$ -interferon, and the activation of transcription factor NF- $\kappa$ B.<sup>30</sup> Additionally, it can induce apoptosis of both macrophages and lymphocytes.<sup>30</sup> The mycotoxin may be a virulence factor in infections caused by *A. fumigatus* and *C. albicans*. The frequency of infections by these organisms in patients with chronic granulomatous disease, who have defects in the oxidative killing of neutrophils, suggests that this mycotoxin is a virulence factor.<sup>31</sup> High levels of gliotoxin have been detected in the serum of animals in models of invasive aspergillosis and in human patients with neoplastic disease and invasive aspergillosis.<sup>30</sup>

The epipolythiodioxopiperazines have been suggested as a potential source for, or a model for the synthesis of, therapeutic agents for several disorders.<sup>29</sup> The disulfide bridge in the toxins imparts an ability to kill cells with increased glutathione levels or with glutathione detoxifying enzymes, as may be seen in some forms of tumor resistance to therapy.<sup>29</sup> Gliotoxin itself has been demonstrated to inhibit an enzyme involved in the function of the *ras* oncogene.<sup>29</sup> This mycotoxin has also been shown to destroy activated hepatic stellate cells, through its ability to induce apoptosis, which are involved in the etiology of the fibrotic process that leads to hepatic cirrhosis.<sup>29</sup>

## Zearalenone (F-2 Toxin)

This mycotoxin is mentioned in this brief overview not because of the significant toxicity associated with its exposure to humans, but because it illustrates the diversity with which mycotoxins act. While the use of most mycotoxins as therapeutic agents is theoretic, zearalenone has been administered for specific effects in both animals and humans. Bennett et al have stated that labeling zearalenone as a mycotoxin is actually a misnomer as, in reality, since its structure resembles 17 $\beta$ -estradiol, it is capable of binding to mammalian steroid receptors and has demonstrated estrogenic effects. It would more properly be labeled a “mycoestrogen” or “phytoestrogen.”<sup>1</sup> Whatever label is given to the substance, a synthetic analog has been used as an anabolic agent in livestock, and the naturally occurring chemical and its synthetic analog have been used to treat postmenopausal symptoms in women.<sup>1</sup>

Zearalenone is produced by *Fusarium graminearum* and several other *Fusarium* species, all of which are found in abundance in association with grains. As such, this has been described as the most common of the mycotoxins associated with *Fusarium* and it has been stated that up to approximately one-quarter of corn consumed by humans contains zearalenone.<sup>32</sup> Pigs are particularly sensitive to its action, and consumption in contaminated feed results in a hyperestrogenic syndrome that may include both infertility and teratogenic effects.<sup>32</sup> The common occurrence of zearalenone and its estrogenic actions have



led to a number of hypotheses related to possible adverse effects on humans, including reduced fertility and stimulation of estrogen-sensitive neoplasms. However, to date, epidemiologic studies have not confirmed any demonstrable adverse effects in humans.<sup>1,32</sup>

## Trichothecenes

This is a very large group of mycotoxins (over 60) and many are extremely toxic. A few of the most toxic include T-2 toxin, deoxynivalenol or vomitoxin, and diacetoxyscirpenol. While chemically heterogeneous, all are sesquiterpenes and all have a 12,13-epoxytrichothene ring.<sup>1</sup> The genus *Fusarium* is the major producer of these substances but other producers include *Trichoderma*, *Myrothecium*, *Phomopsis*, *Trichothecium*, and, importantly, *Stachybotrys* species.<sup>1</sup> Many of these species are plant pathogens and can be associated with food grain or feed contamination. Like many mycotoxins, trichothecenes act by inhibiting protein synthesis, although different trichothecenes act at different stages in the process. As these substances are so potent, and produced by species that grow readily on plants or plant-based materials, they have not only been associated with significant naturally occurring toxicity, but have also been implicated in toxicity that is man-made (bioterrorism) or man-facilitated (sick building syndrome).

## Trichothecene toxicity

Alimentary toxic aleukia is a syndrome that has been attributed to the trichothecenes T-2 and diacetoxyscirpenol. At least 100,000 human deaths during the years 1942–1948 in the former Soviet Union have been blamed on alimentary toxic aleukia, thought to have resulted from eating overwintered grain containing *F. sporotrichoides* and *F. poae*.<sup>1,19</sup> These mycotoxins were never directly identified in the crops but the syndrome has been attributed to the trichothecenes because both species of *Fusarium* have been shown to produce these mycotoxins, the clinical syndrome resembles a similar syndrome in horses which has been shown to be due to trichothecenes produced by *Stachybotrys*, and an experimental syndrome with the same features can be produced in an animal model administered T-2 toxin.<sup>1,32</sup>

In affected patients described in accounts from the Soviet Union, oral mucosal ulcerations and gastroenteritis were followed by pancytopenia due to bone marrow toxicity, with hemorrhage and agranulocytosis.<sup>33</sup> Mortality could be as high as 80% of affected individuals, especially in patients who were also suffering from malnutrition. In many cases, the cause of death was an opportunistic bacterial infection.<sup>32,33</sup>

Although alimentary toxic aleukia has not been reported since the immediate post-World War II period, a syndrome (red mould disease) with some of the components of alimentary toxic aleukia has been reported more recently. This mycotoxin syndrome could be more confidently assigned to trichothecenes produced by *F. graminearum*, using modern analytic techniques.<sup>32</sup> Nausea, vomiting, abdominal pain, diarrhea, chills and headache were the symptoms in a large number of patients with toxicity due to another trichothecene, deoxynivalenol, during 1988 in India.<sup>34</sup> This trichothecene is said to be the most common mycotoxin found in food grains.<sup>1</sup>

Trichothecenes are the major group of mycotoxins thought to be involved in producing a complex set of signs and symptoms, including fatigue, headache, irritation of the eyes, nose and pharynx, a variety of neurologic symptoms such as dizziness and loss of balance, along with cognitive abnormalities such as difficulty in concentrating, and memory loss. This controversial and somewhat poorly defined syndrome has been labeled by a variety of terms, including “toxic mould syndrome,” “sick building syndrome” or “building-related illness,” to name a few. A large array of possibilities in the indoor environment have been postulated to contribute to the etiology of the syndrome, such as organic and non-organic chemicals used in cleaning and construction, poor ventilation, tobacco smoke, noise, even psychosocial factors.<sup>32</sup> Most cogently for this review, mycotoxins have been suggested as a potential cause, as a result of inhaled mycotoxins from mould growing on damp or wet cellulose products, a nutritive source for many moulds and a common component of building materials. This hypothesis was bolstered by the initial findings of extensive *Stachybotrys chartarum* growth in homes of a cluster of children with pulmonary hemorrhage, the demonstration that the isolates produced a variety of potent trichothecenes and the hemolysin stachylysin, and a CDC investigation that suggested a link between the hemorrhage and the mould.<sup>1</sup> A warning to pediatricians issued by the American Academy of Pediatrics about the possible link between mould and the pulmonary hemorrhage contributed to establishing the hypothesis as scientific fact in the perception of many, when in reality, a subsequent CDC report and a number of independent investigations concluded that a definite link between the cases of pulmonary hemorrhage and *Stachybotrys* mycotoxins could not be inferred from the evidence.<sup>1</sup>

The degree of confusion and contradiction surrounding the relationship between the series of infants with pulmonary hemorrhage and mycotoxins is reflective of the entire issue of indoor mould growth, inhalation of mycotoxins by residents of buildings containing mould, and the effects inhaled mycotoxins may have on exposed individuals. An extensive examination of the various aspects of this controversy is beyond the scope of this review. It is clear that there are a number of species of moulds that grow well in damp indoor environments, including *Penicillium* and *Aspergillus* spp. and *S. chartarum*, to name a few. However, as has been pointed out previously, the presence of mould species capable of producing mycotoxins does not automatically imply that mycotoxins will be produced by mould.<sup>32</sup> However, it has been demonstrated that the potential for airborne mycotoxins in a building with heavy growth of mould does exist.<sup>35</sup> If mycotoxins are present in the indoor environment, will they be present in a sufficient concentration to have an adverse toxic effect? The conclusion of the American Academy of Occupational and Environmental Medicine (ACOEM) is that this is unlikely to be the case.<sup>36</sup> Other authors feel that they have demonstrated adverse neurobehavioral and cognitive effects on individuals in environments contaminated with mould, and that mycotoxins produced by the moulds is the most likely cause.<sup>37,38</sup> Many appear to be taking the view put forth by Kuhn and Ghannoum that, while there is suggestive evidence with respect to indoor mould, mycotoxins, and adverse effects on those exposed in the indoor environment, the evidence is not definitive and further study is required.<sup>32</sup>

Another area where trichothecenes appear to hold a unique position among the mycotoxins is with respect to their use as weapons. As has been mentioned in this review, many mycotoxin effects require long-term and additive exposure to become manifest, and in many cases, the cooperation of the subject in ingesting the mycotoxin, since the majority of these substances require an alimentary route of introduction into the human body. These characteristics make most mycotoxins poor choices for weapons, which is why most experts find the stockpiling of aflatoxin for use as a weapon by the former government in Iraq difficult to understand.<sup>1</sup> However, one of the trichothecenes, T-2, has many characteristics that make it an effective weapon. The substance can be absorbed through a variety of routes, including the gastrointestinal, respiratory and dermal route, it acts immediately rather than requiring a long period of additive exposure, and finally, a very small amount, of the order of milligrams, can be lethal.<sup>1,39</sup> It was alleged that T-2 was utilized in south east Asia by the former Soviet Union, as a component of the bioweapon called “yellow rain” (along with nivalenol and deoxynivalenol), although there is great controversy as to whether these mycotoxins, which were detected on foliage analyzed in the United States, were actually components of a weapon or naturally occurring.<sup>1</sup> Another mycotoxin that is not a trichothecene but has been suggested as possible bio-weapon is  $\alpha$ -amanitin from the mushroom *Amanita phalloides*, because of its high toxicity, water solubility, and stability at high temperatures.<sup>39</sup>

## Mycotoxin effects on the human immune system

It has been known for some time that many mycotoxins can influence the immune system of animals, with experiments demonstrating that these substances affect primarily the cellular arm of the immune system.<sup>40</sup> It has been alleged by some who believe that mycotoxins are responsible for the signs and symptoms associated with the “sick building syndrome,” described earlier, that mycotoxins also affect human immune function.<sup>41</sup> That metabolic products of fungi can influence the human immune system is evident, as the unadecapeptide cyclosporine A, which selectively suppresses the function of T cells to prevent many types of rejection and graft-versus-host disease in human transplants, is a substance that was originally detected as a fermentative product of the mould *Tolypocladium niveum*. The effects of gliotoxin on T cell, macrophage, and neutrophil function, and its possible role as a virulence factor in infection, have been previously described in the section on this mycotoxin.

Recently, more studies have begun to examine the influence of some of the mycotoxins described in this short review in human in vitro systems. Aflatoxin has been a favorite because of its consistent inhibitory effect on cell-mediated immunity in a variety of animal models and the potential for studying the in vivo effects of the mycotoxin in humans, as it is one of the few mycotoxins where long-term exposure to populations can be documented by both environmental detection and in vivo biomarkers. Using aflatoxin-albumin as a biomarker, Turner et al examined whether high levels of aflatoxin could be correlated with a variety of tests to assess T cell, B cell, and mucosal immune function.<sup>42</sup> A correlation of high levels of aflatoxin and reduced levels of secretory immunoglobulin A was noted

but the high level of anergy noted in the subjects (50%) could not be correlated with aflatoxin levels. The authors note, however, that such a correlation may require more than the single point in time level that was used in this study.

Using more sophisticated immunologic techniques, other investigators have begun to detect specific defects in immune function in patients chronically exposed to aflatoxin. For example, Jiang et al examined specific characteristics of T cells in the African population tested by Turner et al and were able to correlate a decrease in T cells with an activator inducer molecule (CD69) in subjects with high levels of aflatoxin exposure.<sup>43</sup> The presence of this molecule on a sufficient number of T cells is necessary for a normal immune response to infections. This same study also showed a decrease in effector CD8 T cells that express pore-forming protein and serine proteases, necessary for the killing function of these cells, in those subjects with high aflatoxin exposure.<sup>43</sup> Both these findings suggest the possibility of impaired T cell function in subjects with high levels of aflatoxin exposure.

As biomarkers for other mycotoxins that have been shown to influence immunity in animal models are discovered, the application of modern sophisticated immunologic investigative techniques in future studies should help to clarify the influence of mycotoxins on human immunity.

## Mycotoxins associated with mushrooms

Many reviews do not include mycotoxins produced by mushrooms in their body of work although, as pointed out by Bennett et al, the essential difference between the toxins produced by moulds and those produced by mushrooms is that intoxication with mould-associated toxins results from the unknowing ingestion of contaminated food, while intoxication resulting from mushroom-associated toxins results from the willing ingestion of the poison-containing mushroom, albeit misidentified as an edible non-poisonous species.<sup>1</sup> There are a wide variety of toxins produced by mushrooms, with a variety of clinical effects and a variety of mechanisms of action. An in-depth discussion of even the major ones is beyond the scope of this short review and there are a number of excellent reviews that cover clinical, mycologic, and biochemical aspects.<sup>44-46</sup> Several of these substances bear similarity to some of the mould-associated mycotoxins and will be briefly described here.

Perhaps the most famous, and arguably the most potent, mycotoxin associated with mushrooms is amanitin, a bicyclic octapeptide found in *A. phalloides*. As previously mentioned, it has been suggested as a potential bio-weapon. Within 6–24 hours of ingestion, patients begin to suffer abdominal cramping, nausea and vomiting, and diarrhea, followed by a period when liver damage becomes evident with rising liver enzymes and hyperbilirubinemia, which can progress to liver and renal failure and death during the period of 6–16 days after ingesting the toxin.<sup>44</sup> In some cases, the only life-saving therapy available is liver transplant. Mortality has been approximately 20% in adults and this substance is responsible for over 90% of deaths from mushroom poisoning.<sup>44</sup> Similar to many of the mould-associated mycotoxins, it acts at the level of an enzyme involved in protein synthesis, in this case RNA polymerase II, resulting in cessation of transcription and cell death.<sup>44</sup>

Another mushroom associated mycotoxin that can result in a serious clinical syndrome is orellanine. The mechanism of action is unknown but the toxin bears some structural resemblance to the herbicide paraquat.<sup>45</sup> Paraquat is thought to exert toxicity via redox production of superoxide anions, which can induce lipid peroxidation of membranes and deplete cellular nicotinamide adenine dinucleotide phosphate (NADPH). Whether orellanine's structural similarity to the herbicide also extends to mechanism of action is unknown and requires study. Ingestion of orellanine-containing mushrooms (*Cortinarius* sp.) results in nausea and vomiting with diarrhea 36–48 hours after ingestion, followed by the development of acute renal failure in up to half of patients, which may take up to 3 weeks to manifest.<sup>45</sup> Up to half of these patients may develop chronic renal failure, and dialysis or renal transplant may be required.<sup>45</sup>

Gastrointestinal irritation (gyromitrin), hallucinations (psilocybin), seizures and fluctuating central nervous system effects (isoxazoles), and parasympathetic overstimulation (muscarine) are examples of other syndromes associated with mushroom mycotoxins.<sup>45</sup> Like the mould-associated mycotoxins, there are a wide variety of toxins with an equally broad spectrum of effects on humans.

## Conclusion

While most are familiar with the significant morbidity and mortality associated with the wide variety of mycotic infections that can occur in humans, the same degree of familiarity and concern does not frequently extend to mycotoxins. Due to the difficulty of establishing definitive cause-and-effect relationships between mycotoxins and medical syndromes or disorders, knowledge relating to the pathogenetic potential of the mycotoxins has lagged behind that associated with the mycoses, health officials have not paid sufficient attention to the mycotoxins, and people have suffered and continue to suffer the consequences of exposure. Fortunately, with evolving investigative and epidemiologic techniques, new awareness and interest by the medical and scientific community, and concern on the part of the public, knowledge of the mycotoxins and their medical importance is increasing, which it is hoped will, in turn, lead to better control of exposure and mitigation of their adverse effects. It should be remembered that the fungi that produce these substances are in most cases saprophytic and as such, much more abundant in the environment than the mycoses-causing fungi, with opportunity to affect a larger and more varied population.

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